Influence of Alcohol Consumption on the Autoimmune Process

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The effect of ethanol consumption was investigated in 262 Wistar rats by using several methods of its administration on the development of adjuvant arthritis (AA). The results showed that the self-consumption of 10% alcohol to some degree promotes the development of inflammatory and autoimmune process in rats preferring ethanol, and this effect was more expressed in male rats at the end of experiment. In experiments with intragastric injections of ethanol, the effect on the development of AA depended on the duration of alcoholization. Alcoholization during one week before the reproduction of AA and the subsequent daily injections of it during the whole experiment intensified the autoimmune process, but the same injections of alcohol showed no such effect on the established AA. Direct daily microinjections (10 μl) of 45% ethanol into the lateral brain ventricles intensified the inflammatory process in joints, development of polyarthritis and impaired the blood indices. The use of ethanol solutions with acetylsalicylic acid (ASA) decreased the antiinflammatory action of ASA. Repeated 10% ethanol intragastric injections in combination with ASA led to an increase in plasma beta-endorphin (β-E) level in comparison with control group. Higher ethanol doses decreased plasma β-E and the effect was dose dependent.

Thus, chronic ethanol consumption led to a certain impairment of AA and its action depended on the dose and scheme of alcohol administration. Alterations were greater in the groups that received alcohol infusions intracerebroventricularly.

Key words: ethanol, adjuvant arthritis, autoimmune process

INTRODUCTION

Alcoholism is one of the most important medical and social problems. Ethanol as a very simple molecule easily penetrates various biological membranes and may exert its action by disordering their structure. Thus, it is not surprising that ethanol has a very complex pharmacodynamic profile and that it can interfere with almost every system in the organism. First of all, ethanol acts on the central nervous system (CNS) and causes pathological changes in its regulatory functions and therefore destroys the action of all systems in the organism, acts on organs and tissues and all kinds of metabolism. It is well known that ethanol can exhibit both central stimulatory and inhibitory effects in men as well as in experimental animals. Alcohol exerts numerous pharmacological effects through its interaction with various neurotransmitters and neuromodulators (1). A reduced release of neurotransmitters has been measured in various experimental conditions (2). Ethanol is believed to produce some of its effects through activation of opioid mechanisms (3) and its administration affects the brain and pituitary content of opioid peptides derived from proenkephalin, proopiomelanocortin as well as from prodynorphin. Interaction between ethanol and endogenous opioid systems at the hypothalamic level may be important for explaining the mild analgesic, euphoric and positive reinforcing effects of ethanol (3). Alcohol-induced activation of the opioid system may contribute to the reinforcing properties of alcohol (4). Genetic predisposition toward high alcohol drinking may be associated with an enhanced response of the opioid system to alcohol (5). Recent findings suggest that a genetic deficit in opioid may be a biological marker of alcoholism (6); especially beta-endorphin is a potential biomarker of the genetic risk of alcoholism (7).

Both acute and chronic exposure to ethanol has been shown to alter the function of the hypothala-

mic-pituitary-adrenocortical (HPA) system (8, 9). Acute ethanol administration has been found to increased plasma glucocorticoid concentration, and chronic alcohol intake has been associated with the disruption of circadian rhythms of rodent and human plasma glucocorticoids. In addition, ACTH concentrations have been found to be reduced in hypothalami but not in the pituitary from rats that received 15% ethanol instead of drinking water for 7 days. The influence of alcohol on HPA activation was also inconsistent; some of the animals did not appear to respond even though elevated plasma ethanol levels were produced (10). In rats, acute administration of alcohol induces a dose-dependent increase in plasma ACTH and corticosterone levels. This response depends on the delivery of the hypothalamic peptides, corticotropin-releasing factor (CRF) and vasopressin (VP) to the pituitary gland (11). But the exposure to an alcohol diet for 7 to 10 days significantly blunts the HPA axis in response to immune signals (11). Chronic daily ethanol treatment induced changes in the HPA axis that persisted for at least 3 weeks after complete cessation of ethanol consumption (12). The effects of alcohol on the HPA axis function are biophasic with respect to dosage (inhibition with low doses and stimulation with higher doses) (10). Nitric oxide (NO) participates in the inhibitory action of prolonged alcohol consumption on the HPA axis (13). Ogilvie et al. (11) hypothesized that chronic alcohol treatment might increase the level of NO within the HPA axis and proposed that NO participates in the blunted activity of the HPA axis during prolonged exposure to alcohol.

Alcohol also can weaken or intensify the action of medicines, makes unfavourable conditions and cause significant changes in many pathological processes.

Immunological reactivity is one of the most important systems of homeostasis and its status can define the beginning and the course of diseases. One of the least appreciated medical complications of alcohol abuse is altered immune regulation leading to immunodeficiency and autoimmunity (14). Chronic and even acute moderate alcohol use exerts a profound modulatory effect on the immune system and increases host susceptibility to infections caused by bacterial and viral pathogens (15, 16). Chronic alcoholics often contained circulating autoantibodies. The marked disturbances of the immune system in alcoholics are closely connected with the action of ethanol on the immunocompetent cells, which occur through derangements in the neuroendocrine regulation of immunological processes. An important feature of this response is the interaction between the immune system and the CNS (17, 18). The impaired host defence after alcohol exposure appears to be linked to a combination of decreased inflammatory response, altered cytokine production and abnormal reactive oxygen intermediate generation (15). Cellular immunity, particularly antigen-specific immune response, is impaired by both acute and chronic alcohol use. The functional abnormalities of T and B lymphocytes, NK cells and monocytes/macrophages resulting in an altered immune response seen after alcohol use are widely discussed (15, 19). Alcohol is thought to reduce NK cell responses and to alter cellular immunity by changing the relative balance of Th1 *versus* Th2 cytokine response profiles (20). Free radical formation caused by chronic ethanol administration could activate transcription factors such as nuclear factor–kappa B (NF-κB), which regulates production of inflammatory cytokines (21). Current research on altered cytokine balance produced by alcohol is leading to new insights on the regulation of the immune system in chronic alcoholics (14). Altered cytokine homeostasis has been implicated in the pathogenesis of alcoholic liver diseases. Increased levels of two proinflammatory cytokines, TNFα and IL-6, were observed in these patients (22, 23). These cytokines also play an important role in the pathogenesis of rheumatoid arthritis (RA) (24, 25). Chronic alcoholism complicated by alcoholic liver disease is characterized by activation of the inflammatory response (26). Many alcoholics become seriously deficient in cell-mediated immunity. The primary target of chronic alcohol ingestion is the T lymphocyte. Although T lymphocyte functions can be directly affected by ethanol, the decreased antigen presenting cell function appears to be a key element in the ethanol-induced decrease in cell-mediated immunity (15).

Patients with chronic inflammatory diseases, including RA, have been observed to have $CD57^+$ T expansion in both $CD4^+$ and $CD8^+$ subsets (27). Authors have reported that alcoholic patients also have $CD57^+$ T expansion. The $CD57^+$ T cell subset produces 18- to 30-fold more TNF-α and INF-γ, respectively, than did the $CD57^-$ subset during the first 12 h of stimulation (27).

A limited number of studies have been directed toward determining the effect of chronic ethanol ingestion on the development of RA. Study of the interaction between RA and ethanol consumption has yielded interesting but rather contradictory results. A number of diseases (RA, enteric infections, duodenal ulcer, gallstones, osteoporosis and diabetes mellitus type II) appear to be beneficially modulated by moderate alcohol consumption (28). Alcohol use did not influence the risk of RA in a prospective cohort study of older women (29). Myllykangas-Lousujarvi et al. (30) investigated alcohol-

related deaths in patients with RA. They found 8 alcohol-related deaths among 480 men and 3 deaths among 1186 women with RA and concluded that alcohol either protected from RA or subjects with RA curtailed their drinking after the manifestation of RA.

There are grounds for suspecting that alcohol consumption is a marker or index of high steroid hormone levels (31). RA which is truly partially caused by low levels of hormones may yield spurious suggestions of an ameliorative effect of alcohol.

It is shown (32) that in men, carbohydrate-deficient transferrin (CDT) was associated with RA. This laboratory marker, although most commonly used to assess alcohol misuse, might also serve as a health risk indicator (32).

However, it should be noted that the role of ethanol intake level and its subsequent interaction with the development of RA remains to be defined.

The purpose of this study was to examine the effect of several methods of chronic ethanol administration on the development of adjuvant arthritis (AA) in rats.

MATERIALS AND METHODS

Animals. Six experiments were performed on adult 262 male and female Wistar rats (weight 170–240 g). Animals were purchased from the Bioreglamentas (Vilnius, Lithuania) and housed in cages under standard conditions with free access to normal commercial pellet chow and water prior to the onset of the experiments. They were allowed to acclimate for a few days before being used.

The animal studies were carried out in accordance with the current guidelines for the care of laboratory animals approved by the Institutional Ethical Review Committee.

Induction and evaluation of AA. 0.1 ml of complete Freund adjuvant (Calbiochem, USA) was injected intraplantarly into the footpad of the left hind paw on day 0. To evaluate the progression of the disease two parameters were defined. The swelling of hind paws and the development of polyarthritis in three non-injected paws were determined plethysmographically. Body weight and joint swelling were checked 3 times a week.

Alcohol administration in arthritis rats. Several methods of ethanol administration were used to study the effect of alcohol on the development of AA. Ethanol was diluted from 95% ethanol in distilled water to the concentrations of 10%, 15%, 20%, 25% and 45% which were used in separate experiments.

The ***first experiment*** was performed to determine the development of AA in the alcohol-preferring rats self-drinking 10% ethanol and rats having free choice between alcohol and water. In the first stage of experiment, ethanol-preferring rats were picked up from 100 female and 50 male rats. The rats, housed two per cage, were allowed ad libitum access to 10% ethanol or water and each animal were tested for alcohol preference during two weeks of testing procedure. Water and alcohol were provided in two calibrated 200 ml drinking tubes. In one tube there was a tap water and in the other a 10% solution of ethanol. Daily fluid consumption (both ethanol and water) was monitored. Depending on the preference, 3 groups of animals were picked up: group 1 – animals with an active preference of alcohol (40% of males and 20% of females), group 2 – rats with an intermittent character of alcohol or water preference (20% of females and 20% of males), and group 3 – rats preferring only water and steady against the bent for alcohol (60% of females and 40% of males).

In the second stage of experiment, 60 picked up rats were divided into 6 approximately equal groups (3 male and 3 female groups), and the influence of ethanol on the development of AA was examined. Ten female (group 1) and 10 male (group 4) rats received as a source of fluid a 10% alcohol solution. Groups 2 and 5 consisted of 10 female and 10 male rats and were given a free-choice of drinking between 10% ethanol or water. Groups 3 and group 6 (control) received only water.

The ***second experiment*** was performed on 20 male rats which were randomly divided into ethanol-exposed (group 1) or water-only (group 2). Animals of group 1 received forced intragastric daily (except weekends) injections of ethanol (20% solution). The experiment lasted 17 days.

In the ***third experiment,*** the influence of preliminary alcoholization during one week and subsequent daily intragastric injections of ethanol on the development of AA was investigated on 30 male rats. Group 1 of animals received 10% (dose 0.5 g/kg) and group – 20% (1 g/kg) ethanol solution. Control rats, which were not subjected to the treatment with alcohol were given water alone following an identical protocol. Each of the three groups comprized ten rats.

In the ***fourth experiment*** (50 male rats), various doses of alcohol (10%, 15%, 20% and 25%) were injected intragastrically one week following the AA induction day up to the end of experiment (17th day).

In the ***fifth experiment,*** ethanol was injected into the brain ventricles. Before the intracerebroventricular (i.c.v.) cannulation the animals were selected based on the ethanol consumed during a one-week period of two-bottle choice between 10% ethanol and water. In 52 male rats that preferred alcohol,

plastic cannules with a soldered tip were implanted stereotaxically into the left ventricle of the brain one week before AA induction.

The method of implantation and the microinjection procedures are described in our previous work (33). The animals were housed after surgery in individual cages and were allowed a period of 5 days to recover from surgery before the further procedures were initiated. Then the rats were divided into 3 groups and AA was induced. Group 1 (17 rats) was treated with daily microinjections of 45% alcohol solution and group 2 (18 rats) with microinjections of 20% alcohol solution in a volume of 10 μl per rat. Control group animals were infused via their i.c.v. cannula with an equivalent volume of physiological saline. Solutions were infused slowly over a period of 20 s. Infusions were administered daily (except weekends) for a period of 12 days. At the end of the experiment, only 13 rats of group 1st and 13 rats of group 2 were alive. Three rats of the control group lost their cannulas and were excluded from the experiment. The rat brains were removed and later dissected to determine if the cannula was in the ventriculum. All the animals that survived in the experiment were found to have cannula tips located within the ventricle or on the border of it, so all of the data were retuned for further analysis.

In the ***sixth experiment*** animals, 50 male rats were administered by intragastric injections various doses of ethanol together with acethylsalicylic acid (ASA) since the AA inducing day. The animals were divided into five equal groups: group 1 was treated with ASA (140 mg/kg) and 15% ethanol solution (0.75 g/kg) contained in 1 ml of 1% starch gel, group 2 – with ASA and 20% ethanol (1g/kg), group 3 – with ASA and 25% ethanol (1.25 g/kg), and group 4 – with ASA (140 mg/kg). The control group with AA received intragastric injections of starch gel in the same volume. The experiment lasted 17 days.

At the end of experiments the animals were killed by decapitation preceded by light ether narcosis. Blood was taken for determination of ESR, leukocytes and erythrocyte count (by using a Picoscale, Hungary hematological analyzer). Titres of rheumatoid-like factor (RLF) were determined the using Waaler–Rose reaction. The level of plasma β-endorphin was measured by radioimmunoassay (RIA) (34). Internal organs and joints were examined macroscopically.

Statistics. All animal test groups were compared with control. Student's t test was used to determine the significance ($P < 0.05$) between them. The percentage of deviation from control was derived by the following formula: $(T\text{-}C)/C \times 100$, where T stands for the data on the test group and C on the control.

RESULTS

1. Development of adjuvant arthritis in alcohol-preferring male and female rats

Differences in alcohol and water consumption were almost similar in male and female rats. Rats preferring alcohol consumed greater amounts of a 10% ethanol solution than did rats having a free choice between alcohol and water. The daily mean alcohol intake in alcohol-preferring rats ranged from 166.6 ± ± 13.6 ml per group (6–12 g/kg) in female and 161.3 ± 13.3 ml (3–12 g/kg) in male animals given only 10% alcohol solution and was higher than in groups with free-choice drinking of ethanol. The females of the latter group daily consumed 71.5 ± ± 7.3 ml (3–6 g/kg) of alcohol and 182.5 ± 16.1 ml (8–12 g/kg) of water and males 61.3 ± 6.2 ml (2–4 g/kg) of ethanol and 189.1 ± 10.45 ml (6–9 g/kg) of water. Control groups consumed daily up to 200 ml (11–12 g/kg – females and 8–9 g/kg – males) of water.

The results of investigation (Fig. 1) showed that neither alcohol alone nor a free choice between alcohol or water exerted a significant action on joint swelling. Some increase in females preferring alcohol was observed in the first part of experiment (days 3, 8 and 12). In females having free choice between alcohol solution and water this tendency was observed till day 8. In males preferring ethanol, some increase of joint swelling in comparison with control group was revealed on days 5, 12 and 17 (*i.e.* to the end of experiment) and in animals having a free-choice between alcohol or water on day

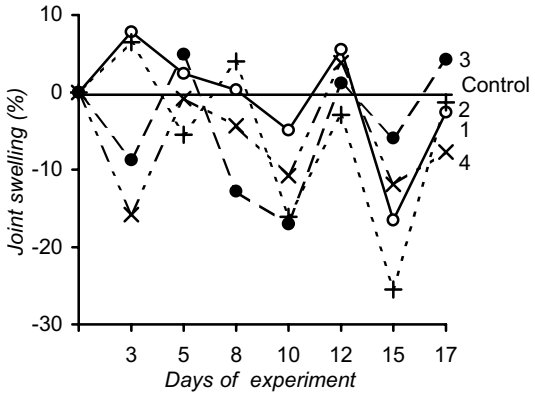

Fig. 1. Influence of ethanol self-consumption on joint swelling in alcohol-preferring male and female rats with adjuvant arthritis (mean indices are expressed as percentages from mean indices in arthritic control).
1 – female rats self-drinking 10% alcohol solution; 2 – female rats having a free choice between alcohol and water; 3 – male rats self-drinking 10% alcohol solution; 4 – male rats having a free choice between alcohol and water

12. Polyarthritis developed only in 10% of male rats of the both test groups.

There were no essential differences in body weight between the test and control groups, except a slight decrease (by 11–13%), especially in male rats that consumed ethanol solution. In female rats and male rats preferring ethanol, significantly decreased the liver, spleen and thymus weight and in male rats that had a free choice between alcohol solution and water also decreased the weight of kidney and adrenal gland weight in comparison with control groups (Table 1). ESR and the count of leukocytes were higher in all test groups, except female rats offered a free choice between alcohol and water, but a significant increase was observed only in male rats preferring ethanol (group 4). The count of leukocytes was also higher in male rats preferring alcohol and having a free choice between ethanol and water. Titres of RLF expressed in $\log_2$ were higher in female (1.90 ± 0.47) and male rats (2.3 ± ± 0.36) preferring ethanol, but a significant difference in comparison with control groups (1.20 ± ± 0.38 in females and 1.10 ± 0.45 in males) was observed only in the male group ($P < 0.05$).

Macroscopic examination of internal organs revealed lung changes (brown spots) in 30% of females of both test groups and in 20% of male rats preferring alcohol, as well as in 10% of rats that had a free choice between alcohol and water. In control animals, only 10% had affected lungs.

Thus, self-consumption of alcohol to a certain degree promotes the development of inflammatory and autoimmune process in rats with AA.

2. Effect of forced injections of ethanol on the development of AA

Daily intragastric injections of 20% ethanol (dose, 1 g/kg) in male rats during two weeks since the AA inducing day produced a more pronounced inflammatory effect throughout the whole experiment. Joint swelling was greater (by 16–20% on days 5, 8, 12 and 15) in comparison with control group (Fig. 2(1)). Polyarthritis characterizing the develoment of the autoimmune process developed in 40% of rats given alcohol and in 20% of ani-

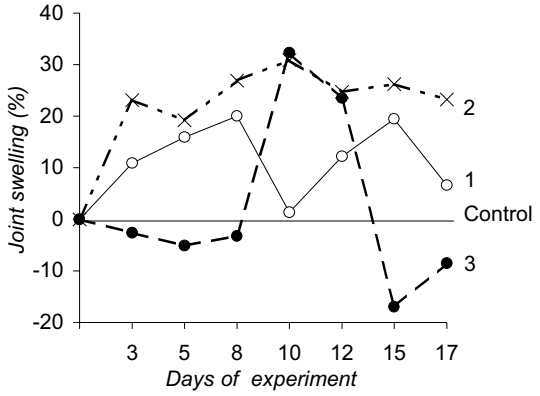

Fig. 2. Joint swelling in male rats with AA treated with intragastric injections of 20% ethanol solution (mean indices are expressed as percentages from mean indices in arthritic control). 1 – alcoholization since AA inducing day; 2 – preliminary (during one week) and subsequent alcoholization till the end of experiment; 3 – alcoholization since day 8 of AA.

Table 1. **Effect of ethanol intake on body and organ weight and peripheral blood hematological indices in male and female rats with adjuvant arthritis**

Groups	Weight						Blood indices	
	Body (g)	Liver (g)	Kidney (g)	Spleen (g)	Thymus (mg)	Adrenal glands (mg)	ESR (mm/h)	Leukocytes (10^9 L)
Females								
1	173.63 ± 3.02	6.62 ± 0.09 P < 0.05	1.46 ± 0.02	0.86 ± 0.03 P < 0.001	0.190 ± 0.02 P <0.01	55.36 ± 2.62	12.18 ± 1.97	14.0 ± 0.54
2	187.0 ± .48	6.92 ± 0.23	1.45 ± 0.04	1.08 ± 0.09	0.239 ± 0.03	56.80 ± 1.88	9.90 ± 2.17	12.19 ± 0.92
3	182.0 ± 3.26	7.14 ± 0.22	1.45 ± 0.04	1.08 ± 0.09	0.298 ± 0.02	55.5 ± 2.48	10.10 ± 1.40	12.79 ± 0.25
Males								
4	225.5 ± 9.64	7.96 ± 0.46 P < 0.01	1.84 ± 0.08	1.07 ± 0.10 P < 0.02	0.219 ± 0.01 P < 0.05	55.90 ± 3.76	18.00 ± 3.43 P < 0.05	13.38 ± 0.54 P < 0.05
5	221.5 ± 7.52	7.40 ± 0.34 P < 0.001	1.78 ± 0.04 P < 0.02	1.15 ± 0.10 P < 0.05	0.210 ± 0.01 P < 0.02	49.30 ± 1.85 P < 0.02	14.10 ± 2.77	14.92 ± 0.32 P < 0.001
6	248.5 ± 11.47	9.55 ± 0.22	1.94 ± 0.04	1.47 ± 0.12	0.260 ± 0.01	61.20 ± 4.25	9.10 ± 1.85	11.83 ± 0.46

Note. Differences are significant in comparison with respective control groups. Groups 1 and 4 – 10% ethanol; groups 2 and 5 – 10% ethanol or water; groups 3 and 6 – control (water).

mals in the control group. Forced alcohol injections significantly ($P < 0.05$) increased the liver weight (8.34 ± 0.32 g in tested group and 7.45 ± ± 0.24 g in control). ESR was also somewhat higher (by 8.6%) in the alcohol-fed group (24.22 ± ± 5.17 mm/h and 22.3 ± 2.13 mm/h in test and control groups, respectively).

It should be noted, that the effect of alcohol on joint swelling depended on the duration of alcoholization. If in the case of preliminary intragastric injections of the same dose of ethanol during one week and subsequent daily injections since the AA inducing day increased the intensity of joint swelling (Fig. 2(2)), alcoholization of established AA (during 7 days) showed an opposite effect, especially at the end of experiment, where joint swelling somewhat decreased in comparison with control group (Fig. 2(3)).

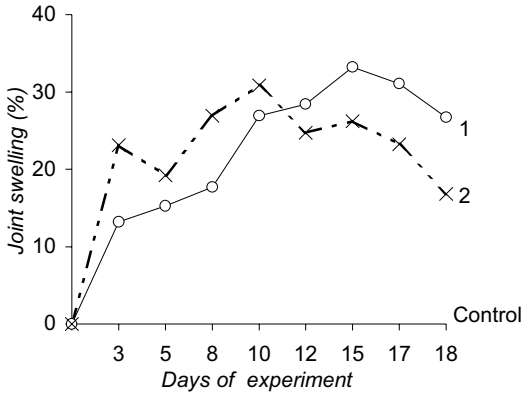

Fig. 3. Effect of preliminary and subsequent intragastric injections of ethanol solutions on joint swelling in rats with adjuvant arthritis (mean indices are expressed as percentages from mean indices in arthritic control). Preliminary alcoholization during one week before the induction of AA. Subsequent treatment with alcohol since AA inducing day till the end of experiment. 1 – 10% ethanol solution; 2 – 20% ethanol solution.

3. Effect of preliminary and subsequent forced intragastric injections of ethanol on the development of AA

In experiment, two groups of rats were treated by intragastric injections of 10% or 20% ethanol solution (dose, 0.5 g/kg and 1 g/kg). The results (Fig. 3) showed that preliminary (during one week) and subsequent forced injections of ethanol till the end of experiment evoked some increase of inflammatory process in joints, as compared with the control group. No essential changes in joint swelling depending on alcohol dosage were observed. Both doses of ethanol were found to intensify insignificantly joint swelling during the whole experiment. Joint swelling in the first stage of AA (till day 10) was by 23–30.8% greater than in the control group under treatment with higher doses of ethanol. In the second stage of AA (12th–18th days of experiment), 10% ethanol solution was more effective and increased joint swelling (by 27–33%) in comparison with control group. The development of polyarthritis was observed in 30% and 40% rats given 10% and 20% alcohol solutions, respectively, *versus* 20% in the control ones.

ESR and leukocyte count in rats treated with 17 intragastric injections of 20% ethanol was higher by 12.4% and 24.3%, respectively, and the amount of erythrocytes was lower by 8.9% ($P < 0.01$) than in control animals (Table 2). Both doses of alcohol significantly increased RLF titres expressed in $\log_2$ (1.3 ± 0.21 in group 1; $P < 0.05$; 1.4 ± 0.16 in group 2, $P < 0.01$; and 0.5 ± 0.24 in control). Body weight did not differ essentially among the groups but was lower by 3–4% in the test groups.

Investigation of internal organs showed a significant decrease of liver and kidney weight in rats of group 1 which received 10% ethanol.

Table 2. **Effect of preliminary and subsequent ethanol injection on body and organ weight and peripheral blood hematological indices in male rats with adjuvant arthritis**

Groups	Weight					Blood indices		
	Body (g)	Liver (g) (g)	Kidney (g) (g)	Spleen (g) (g)	Thymus (mg)	ESR (mm/h)	Leukocytes (10^9 L)	Erythrocytes (10^{12} L)
1 (10% ethanol)	225.0 ± 7.6	7.85 ± 0.33 P < 0.05	1.61 ± 0.07 P < 0.01	0.96 ± 0.10	268.0 ± 0.03	12.40 ± 2.21	9.62 ± 0.39	4.77 ± 0.17
2 (20% ethanol)	227.5 ± 9.19	8.81 ± 0.40	1.80 ± 0.05	1.09 ± 0.05	325.0 ± 0.03	14.10 ± 2.77	11.71 ± 0.77	4.52 ± 0.11 P < 0.02
3 control (water)	234.4 ± 7.38	9.03 ± 0.38	1.86 ± 0.04	1.19 ± 0.06	328.0 ± 0.02	12.55 ± 2.61	9.42 ± 0.89	4.96 ± 0.12

Note: Differences are significant in comparison with control group.

4. Development of AA under subsequent injections of ethanol in various doses

There was no difference in joint swelling up to day 8 (the test day on which all groups of rats received various doses of intragastric injections of ethanol) among the groups with AA (Fig. 4). Since day 8, rats with AA showed a dose-dependent response to alcohol: higher doses (1 g/kg and 1.25 g/kg of ethanol) resulted in a higher short-term joint swelling as compared with control group. The peak of joint swelling was observed on day 10, *i.e.* after two injections of ethanol. In the first two groups joint swelling exceeded control by 9.8% and 11.6%. In group 3, which daily received 1 g/kg of ethanol, the increase of joint swelling from control was 32.3% and in group 4 (ethanol dose 1.25 g/kg) it significantly differed from control ($P < 0.02$) and exceeded it by 43.5%. Increase of joint swelling in both latter groups by 22.2–23.5% was also observed on day 12. At the end of experiment, only intragastric ethanol injections in the highest dose were found to have intensified joint swelling insignificantly. This index exceeded the control by 10.05% (Fig. 4(4)). In other test groups, suppression of joint swelling was observed at the end of experiment (on day 17), especially in groups 1 and 2, where it significantly differed from the control group.

Polyathritis developed in 20% rats of group 1, 10% in group 2, 40% in groups the 3 and 4, and 50% in the control group.

There were no essential differences in body and organ weight, blood indices and titres of RLF among the test and control groups, except spleen weight, which was significantly ($P < 0.01$) lower in rats (gr. 2) that received 15% alcohol solution in a dose 0.75 g/kg (1.14 ± 0.05 g in the test group and 1.45 ± 0.08 in control). ESR increased by 30% (13.0 ± 2.38 mm/h) in rats that received 20% ethanol and by 7% after 25% ethanol injections (10.7 ± 1.77 mm/h in test groups and 10.0 ± ± 1.46 mm/h in control).

Macroscopic investigation of internal organs showed the injured spleen (covered with whitish spots) in 20% animals of the groups 3 and 4 and in 10% of the control group were found.

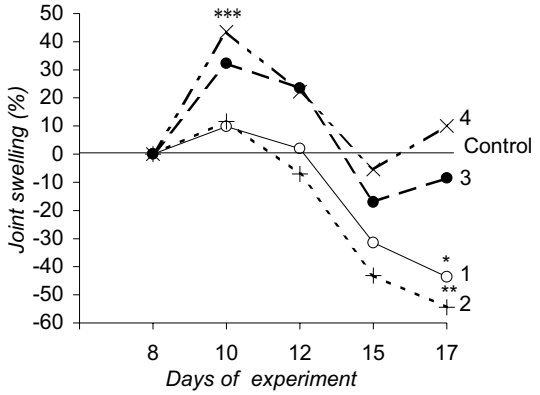

Fig. 4. Effect of intragastric injections of joint swelling in male rats with established adjuvant arthritis (mean indices are expressed as percentages from mean indices in arthritic control).
Duration of intragastric injections 8 days.
1 – 10% ethanol solution; 2 – 15% ethanol solution; 3 – 20% ethanol solution; 4 – 25% ethanol solution.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.02$ in comparison with control group.

5. Effect of intracerebroventricular (i.c.v.) injection of alcohol solutions on the development of AA in rats

As one can see in Fig. 5, the i.c.v. microinjections (10 μl) of alcohol solutions (45% and 20%) exepted a pronounced inflammatory eddect.

Joint swelling in rats of group 1 given 45% ethanol solution was higher on the average by 11–13% in comparison with the control group on days 3, 10, 12. An especially pronounced increase of this index was observed at the end of experiment (15 and 17 days), where joint swelling exceeded control by 40.2% and 48.6% ($P < 0.05$).

Microinjections of 20% ethanol solution during the first stage of investigation (till day 12) had a more evident inflammatory effect. A pronounced increase in joint swelling was revealed on days 3 ($P < < 0.02$) and 5 ($P < 0.05$) and exceeded the control by 19–23.5% during the whole experiment.

The incidence of polyarthritis was observed in 46% of animals of group 1, 17% of group 2 and 27% of group 3.

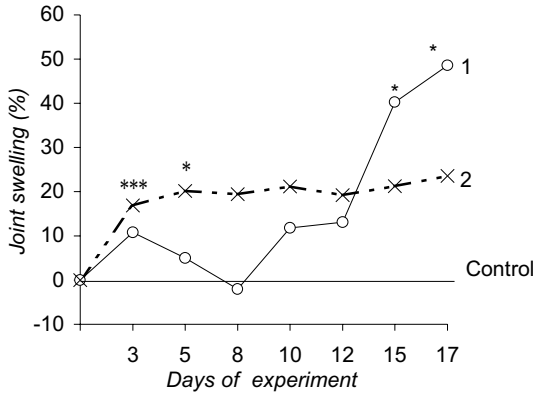

Fig. 5. Effect of intracerebroventricular microinjections of ethanol on joint swelling in male rats with AA (mean indices are expressed as percentages form mean indices in arthritic control).
1 – 45% ethanol; 2 – 20% ethanol.
* $P < 0.05$; *** $P < 0.02$ in comparison with control group.

There was no essential difference in body weight among the groups. 45% alcohol microinjections significantly ($P < 0.05$) diminished the weight of kidney (0.83 ± 0.02 g in the test group and 0.90 ± 0.02 in control) and increased ESR (by 11.7%) and leukocyte count (by 30.3%; $P < 0.01$) in comparison with the control group (15.56 ± 0.82 × 10^9 in the test group and 11.94 ± 0.79 × 10^9 L in control).

RLF titres (expressed in $\log_2$) were insignificantly elevated in groups 1 (by 27.06%) and 2 (14.68%) given ethanol microinjections i.c.v.

It should be noted that during the experiment 4 rats of group 1 and 5 rats of group 2 perished. Autopsy of these animals showed no macroscopic changes either in internal organs or in the brain, except one rat of group 1, in which affected lungs (brown colour) and haemorhages in the intestine and two rats of group 2 with affected lungs were observed.

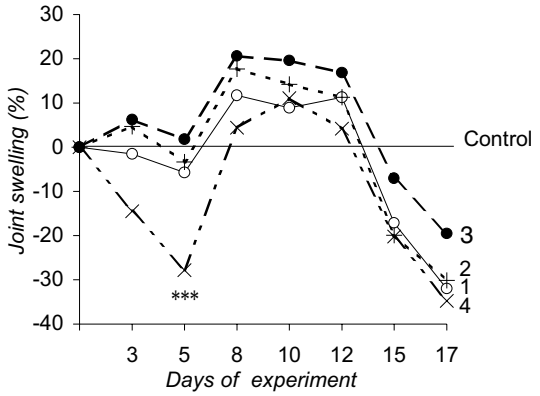

Fig. 6. Joint swelling on administration of various doses of ethanol in combination with acetylsalicylic acid (ASA) in male rats with adjuvant arthritis (mean indices are expressed as percentages from mean indices in the arthritic control).
1 – ASA (140 mg/kg) and 15% ethanol; 2 – ASA (140 mg/kg) and 20% ethanol; 3 – ASA (140 mg/kg) and 25% ethanol; 4 – ASA (140 mg/kg); 5 – control (starch gel). ***$P < 0.02$ in comparison with control group.

6. Effect of intragastric injections of various doses of ethanol in combination with acetylsalicylic acid (ASA) on the development of AA

The effect of combined treatment with alcohol and ASA on joint swelling is shown in Fig. 6. Neither ASA alone (exept day 5) nor its combination with alcohol solutions exerted a significant action on joint swelling, although almost all measurements under the usage of ASA in combination with alcohol showed an aggravation of inflammatory process till day 12. At the end of experiment the treatment with ASA diminished joint swelling by 20.2% (day 15) and 34.78% (day 17). ASA in combination with the highest dose of ethanol (1.25 g/kg) showed the peak of joint swelling in comparison with the other test groups. It was lower than in control by 19.9%, whereas in groups 1 and 2 joint swelling was diminished by 30.16% and 32%.

Polyarthritis developed in 20% rats of groups 1 and 2, 30% in group 3 and 10% in group 4 (ASA). In the control group, polyarthritis was found in 50% of animals. The body weight at the end of experiment was slighly lowered in all test groups (Table 3), but this decrease was most pronounced in

Table 3. **Body and organ weight, blood indices and plasma β-endorphin level in rats with AA treated with alcohol solutions in combination with acetylsalicylic acid (ASA)**

Groups	Weight					Blood indices			
	Body (g)	Liver (g)	Kidney (g)	Spleen (g)	Thymus (g)	ESR (mm/h)	Leukocytes (10^9 L)	Erythrocytes (10^{12} L)	Plasma β-E (pmol/L)
1 ASA+15% alcohol	210.5 ± 13.81	6.90 ± 0.43	1.66 ± 0.09	0.97 ± 0.13	0.200 ± 0.02	14.60 ± 2.21	9.40 ± 0.78	5.77 ± 0.23	29.70 ± 7.06
2 ASA+20% alcohol	205.5 ± 9.23	6.49 ± 0.31 $P_{5-2} < 0.02$	1.50 ± 0.04 $P_{5-2} < 0.02$ $P_{4-2} < 0.001$	0.72 ± 0.04 $P_{5-2} < 0.05$ $P_{4-2} < 0.001$	0.185 ± 0.02	12.80 ± 2.32	10.27 ± 1.46	6.29 ± 0.16	14.10 ± 3.18
3 ASA+25% alcohol	195.5 ± 11.01	6.88 ± 0.48	1.53 ± 0.07 $P_{4-3} < 0.01$	0.76 ± 0.06 $P_{4-3} < 0.01$ $P_{5-3} < 0.05$	0.138 ± 0.01 $P_{4-3} < 0.01$ $P_{5-3} < 0.01$	12.55 ± 3.15	9.00 ± 0.63	6.69 ± 0.18	4.38 ± 0.65 $P_{5-3} < 0.02$
4 ASA	210 ± 6.87	7.54 ± 0.19	1.77 ± 0.04	1.05 ± 0.06	0.203 ± 0.02	9.71 ± 2.32	10.56 ± 1.08	6.19 ± 0.13	11.83 ± 5.14
5 control	220.0 ± 9.01	7.93 ± 0.47	1.75 ± 0.09	0.99 ± 0.11	0.189 ± 0.01	15.5 ± 2.69	10.31 ± 0.61	6.35 ± 0.13	12.45 ± 2.84

Note. Differences are significant in comparison with groups 4 and 5.

group 3 given ASA with 25% ethanol solution. Significant differences during experiment were observed only on day 8 (191.0 ± 7.44 g in the test group and 218.5 ± 10.72 g – control; $P < 0.05$) and on day 15 (203.0 ± 7.60 g – 3rd group and 232.0 ± ± 9.86 g in control; $P < 0.05$).

The weight of liver, kidney and spleen was significantly lower in group 2, of spleen and thymus in group 3. A decrease in kidney and spleen weight was also observed in animals of groups 2 and 3 and in thymus weight of the latter group in comparison with group 4 given only ASA (Table 3). In the animals that received ASA with ethanol solutions a higher ESR as compared with the group given only ASA was revealed.

No essential differences were observed in RLF titres. Its level was highest in the animals of group 3 that received 25% ethanol in combination with ASA (2.77 ± 0.61 in the test group and 2.00 ± ± 0.35 in control).

The highest level of plasma β-E was found in rats given 15% ethanol in a dose 0.75 g/kg with ASA and the differences were significant in comparison with group given ASA and control ($P < 0.05$). The increase of ethanol dose promoted a decrease of β-E level (Table 3), and the lowest level was observed in rats given ASA with 25% ethanol. So, if the lower doses of ethanol (0.75 g/kg) increased the β-E level, the higher doses (1.25 g/kg) had an opposite effect.

DISCUSSION

The results of our experimental investigation showed that forced and semi-forced consumption of ethanol and its direct microinjections into the cerebral ventricles to a certain degree impaired the development of AA in rats.

AA is a T-lymphocyte-dependent immune-mediated disease. The influence of alcohol on the immunocompetent system is maybe an important mechanism to determine the course of AA. Chronic alcohol intoxication, after a short-term stimulation of protective mechanisms, induces a suppression of many factors of native immunity and decreases resistance of experimental animals to infections and toxins. The injury of the immune system during alcohol consumption is connected with the direct action of alcohol on immunocompetent cells and with the disturbances in the neurohumoral regulation of immunological processes. Long-term consumption of alcohol disturbs the mechanisms of nonspecific immunity, the function of macrophages, diminishes the count of T-lymphocytes and increases the functional activity of B cells. A preferential induction of Th2 *versus* Th1 immune response has been suggested, based on the increased immunoglobulin levels seen in chronic alcoholics. Alcohol is thought to reduce NK cell responses and to alter cellular immunity by changing the relative balance of Th1 *versus* Th2 cytokine response profiles (20).

Immune system activation during AA leads to increased circulating levels of IL-1, IL-6 and TNF-α (24, 25, 35, 36). Alcoholics have been shown to exhibit an increased susceptibility to infections. Chronic alcohol treatment impairs host response to bovine mycobacterium infection from BCG (16), which is also used in complete Freund's adjuvant. BCG infection significantly increased TNF-α, IFN-γ and IL-10 mRNA. Chronic ethanol plus BCG infection further increased TNF-α and IFN-γ. It has been shown that patients with chronic inflammatory diseases, including RA, and alcoholic patients have $CD57^+$ T cell expansion in both $CD4^+$ and $CD8^+$ subsets, and this $CD57^+$ T cell subset produced 18- to 30-fold more TNF-α and IFN-γ than did the $CD57^-$ subset (27).

Thus, ethanol enhances the inflammatory and autoimmune processes during AA development by inhibiting the protective reactions of the organism, in which the CNS and the immunocompetent organs play an important role (18), and aggravates the course of a pathological process.

AA is associated with a number of neuroendocrine changes (37) and results in chronic activation of HPA axis. Alcohol administration also activates the HPA axis in both male and female rats, with females secreting more adrenocorticotrophin (ACTH) and corticosterone than males in response to the same dose of alcohol (38). However, although alcohol intoxication activates the HPA axis and results in elevated glucocorticoid levels (39), under chronic alcohol consumption tolerance may develop to HPA axis-activating effects of alcohol.

Interestingly, equivalent consumption of alcohol (on a weight basis) does not produce equal pathology in man and rodent. With a more rapid metabolism, rats can consume a much greater quantity of alcohol without functional impairment or damage to vital organs. To find clearcut alcohol-induced differences in most organs, alcohol should be given for less than 6 months.

It should be noted that alcohol in our experiment was administered for a short period – only till the stage of polyarthritis. So, in the 1st experiment (semi-forced alcoholization or free choice between alcohol and water) alcohol was given to drink for 26 days and in the 2nd experiment (forced alcoholization) for 12 days. In the 3rd experiment, where treatment with alcohol was used before and during AA induction, alcoholization lasted 17 days, and in the 4th experiment alcohol was injected into the lateral ventricles of the brain for 12 days.

Selection of animals preferring alcohol showed that 20% of females and 40% of males preferred ethanol. This was in agreement with the observations of other authors who showed a faster formation of alcohol motivation in males. There was no significant gender-related differences in the rate of ethanol consumption in the experiment with self-drinking of alcohol. In the animals that preferred alcohol, when they were given 10% alcohol as the only liquid for drinking, a more pronounced effect and a slightly intensified inflammatory and autoimmune process was observed in male rats at the end of experiment. In groups with a free choice of alcohol or water, differences were less expressed.

In experiments with intragastric injections of ethanol, its effect on AA development was dependent on the duration of alcoholization. Alcoholization during one week before AA reproduction and the subsequent daily injections of alcohol during the whole experiment intensified autoimmune process, but the same injections of alcohol showed no such effect on the established AA where the duration of alcoholization was only 7 days.

Direct injections of lower and higher doses (20% and 45% ethanol) of alcohol into the lateral ventricle of the brain to rats with AA markedly intensified inflammatory response, increased joint swelling, changed for the worse the indices of pathological processes. At the end of experiment this effect was dose-dependent.

Weight decrease in the body and various organs (liver, kidney, spleen, thymus) was observed nearly in all experiments. Maybe it was related with a decrease in chow use. However, Meadows and coauthors (40) observed that ethanol induced gross alterations in the peripheral blood, spleen and thymus of ethanolfed mice. The spleen is a major lymphoid organ, where B and T lymphocytes constitute the major cellular components. Following administration of ethanol there was an acute decrease in the size and cellularity of the spleen (a loss of B lymphocytes, with no alteration in $CD4^+/CD8^+$ ratios). Thymus weight was also lower in many of our test groups, which is in agreement with the observations of other authors (12) where ethanol introduction followed by 4 weeks of chronic daily ethanol consumption significantly increased plasma corticosterone levels, adrenal gland weight and decreased thymus and spleen weights in rats.

The liver also underwent significant morphological changes following ethanol treatment; histological examination revealed the accumulation of large intra-hepatic fat deposits and limited necrosis (41). Hepatic necrosis in ethanol-consuming rats is accompanied by an increase in plasma levels of IL-6 and TNF-α (42). Our previous studies also showed alterations in hepatic parenchyma, inflammatory infiltration of hepatic stroma and expressed fibrotic processes under acute treatment with ethanol (43). It is likely that fibrosin, novel fibrogenic cytokine, which may be derived from inflammatory cells, contributes to alcohol-induced hepatic fibrosis in vivo (44).

Our experiments showed that in the animals that preferred ethanol, lungs were often damaged (in 30% of females and 20% of males).

Ethanol interacts with cell membranes diffusely in the brain and produces a similar but more widespread depression of cell response than do the opiates. As mentioned above, i.c.v. microinjections of ethanol solutions intensified the inflammatory process in joints, probably because of a direct influence of ethanol on brain structures, especially on the hypothalamus; besides, alcohol, when administered i.c.v. at high doses, might cause neuronal death. Hypothalamic β-E has been reported to increase, decrease and remain unchanged after chronic ethanol intake (45). One of the ways in which ethanol may affect opioidergic neurons in the hypothalamus is its influence on CRF-containing neurons in this structure (3). It appears that hypothalamic CRF, via its action on the HPA axis, is involved in the reinforcing effects of alcohol (46). Several lines of evidence suggest that ethanol interacts with brain opioid systems and inhibits the binding of ligands to mu(μ)- and delta(δ)-opioid receptors. The inhibition in binding of μ and δ opioid ligands by ethanol involves modification of the conformation of both the receptors and the ligands (47). Findings from animal studies indicated that ethanol-preferring mice (analogues to the high risk human subjects) had higher levels of hypothalamic β-E activity than did ethanol-avoiding mice (analogues to the low risk human subjects) under basal conditions (48). In ethanol-preferring animals (mice, rats) the increased release of β-E following exposure to ethanol was associated with a higher density of δ- or μ-opioid receptors in brain regions important for reinforcement. As shown in (49), repeated ethanol consumption increased β-E, but had no effect on met-enkephalin or α-neoendorphin levels in the hypothalamus. Ethanol increased the plasma level of β-E-related peptides in high-risk but not in low-risk subjects in a dose-dependent manner (50), what indicates that the pituitary β-E system but not the adrenal cortisol system of the high risk subjects shows an enhanced sensitivity to ethanol, which may be an important factor in controlling ethanol consumption. Since in the pituitary gland β-E and ACTH are produced from the same precursor molecule, pro-opiomelanocortin, it may be expected that alterations in plasma ACTH and cortisol levels should induce parallel changes in plasma β-E levels (51). In our study, repeated 10%

ethanol intragastric injections with ASA in rats with AA led to an increase in plasma β-E level in comparison with control group. A decrease of this level was observed under higher doses of ethanol and the effect was dose-dependent. This finding is in agreement with the observations that in higher alcohol preference rats the metabolic rates and functional activity are lower. Carter and Soliman (52) showed that ethanol administration significantly decreased both β-E and met-enkephalin levels in the hypothalamus of rats.

Changes in the development of AA under injections of various doses of alcohol may be caused in part by changes in the synthesis/release of CRF, possibly under the influence of NO. CRF, VP, ACTH and corticosteroids are important regulators of the immune system, behavior, metabolic pathways. Alcohol therefore may influence such functions through a pathological secretion of these hormones. Simultaneous hyperfunction of hypophyseal ACTH cells and ACTH-dependent zones of the adrenal cortex in chronic alcoholics proves that alcohol primarily acts at the level of hypothalamus and hypophysis, while adrenals react to hypersecretion of ACTH cells (53).

Keates et al. (54) showed a high density of opioid binding sites found in the inflamed articular and periarticular tissues. Alcohol penetrates into the synovial fluids, where it can serve as one of the available biological specimens for the prediction of blood and urine alcohol concentrations (55). In our previous experiments where acute toxicity of ethanol in rats (43) was investigated, there were observed expressed edema, synovium villi proliferation, fibrosis and angiomatosis, cartilage erosion and thinning. So, alcohol potentially increased the manifestations of arthritis.

Alcohol in our experiments impaired the antiinflammatory action of ASA, maybe because of the synthesis of prostaglandins (PG) which suppressed ASA. Ethanol was shown to influence the peripheral metabolism of PG (56).

In summary, our findings support the idea that impairment of AA in animals under chronic consumption of alcohol is related, at least partially, to functional alterations of immune reactions and the influence on CNS. The results of six experiments allowed us to draw the following conclusions.

• Application of ethanol to rats decreases the resistance of the organism during the course of adjuvant arthritis, intensifies autoimmune reaction and joint injury.

• In female rats preferring alcohol, joint swelling was increased during the first stage of experiment and in male rats at the end of it. Intensification of autoimmune reactions was also observed in the latter. The weight of organs (liver, spleen, thymus) decreased in both sexes of animals given ethanol.

• Forced repeated injections of 20% ethanol into the stomach intensify joint swelling and polyarthritis development.

• Various doses of ethanol before inducing AA and during its course increased joint swelling, polyarthritis development and reduced the weight of internal organs.

• Daily injections of ethanol into brain ventricles markedly increased joint swelling, leukocytes count and polyarthritis development.

• An application of ethanol solutions with acetylsalycilic acid (ASA) impaired the antiinflammatory action of ASA, significantly lowered the weight of liver, kidney and spleen.

• The level of plasma β-endorphin decreased in connection with the increased doses of consumed ethanol. Low doses of alcohol elevated the level of β-endorphin and high doses showed an opposite effect.

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ALKOHOLIO POVEIKIS AUTOIMUNINIAM PROCESUI

S a n t r a u k a

Bandymuose su 262 Wistar veislės žiurkėmis buvo ištirtas alkoholio poveikis adjuvantinio artrito eigai. Gauti duomenys parodė, kad pusiau priverstinė alkoholizacija, kai žiurkės pačios gerdavo 10% alkoholį, nedaug sustiprino uždegiminį ir autoimuninį procesą žiurkėms, bevelyjančioms etanolį, ir šis efektas bandymo pabaigoje buvo ryškesnis patinams. Taikant priverstinę alkoholizaciją (alkoholio įšvirkštimą į skrandį) poveikis į AA priklausė nuo alkoholizacijos trukmės. Alkoholio taikymas savaitę prieš AA sukėlimą ir bandymo metu sustiprino autoimuninį procesą, kai ta pati alkoholio dozė, taikoma jau išsivysčiusio artrito metu, nesukėlė tokio efekto. Kasdieninės 10 µl 45% alkoholio injekcijos į lateralinį smegenų skilvelį sustiprino sąnarių uždegimą, poliartritų atsiradimą ir kraujo rodiklių pablogėjimą. Alkoholio tirpalai, taikomi kartu su acetilsalicilo rūgštimi (ASR), sumažino pastarosios priešuždegiminį poveikį. Plazmos beta-endorfino (β-E) kiekis, palyginus jį su kontrole, padidėjo nuo 10% etanolio injekcijų, taikytų kartu su ASR. Didesnės etanolio dozės mažino nuo dozės priklausomą plazmos β-E kiekį.

Taigi ilgalaikis etanolio taikymas šiek tiek pablogino AA eigą, priklausomą nuo alkoholio dozės ir taikymo schemos. Didesnius neigiamus AA pokyčius sukėlė alkoholio infuzijos į smegenų skilvelius.

Raktažodžiai: etanolis, adjuvantinis artritas, autoimuninis procesas