Effect of Caffeine on Mouse Immunocompetent Cells

Almira Ramanavičienė[1], Juzefa Ačaitė[1], Aldona Dringelienė[1], Arvydas Markevičius[1], Arūnas Ramanavičius[2,3]

[1] *Laboratory of Ecological Immunology, Institute of Immunology of Vilnius University, Vilnius, Lithuania*
[2] *Sector of Immunoanalysis and Informatics, Institute of Immunology of Vilnius University, Vilnius, Lithuania*
[3] *Department of Analytical and Environmental Chemistry, Vilnius University, Vilnius, Lithuania*

This is a report on the potential influence of caffeine on some immune parameters (spleen index, the concentration of lymphocytes, basophiles, monocytes, neutrophils, and eosinophils) of mice. During the present study mice were divided into five groups 4 mice in each. Group 1 (control) was given water; group 2 was given 2 mg/kg caffeine; group 3–20 mg/kg caffeine, group 4–40 mg/kg caffeine, group 5–200 mg/kg caffeine solution in water by oral intubations. Caffeine solutions were given at 3-day intervals during 30 days. In group 3, a significant increase in spleen weight was detected and the spleen index was 2.0 times ($p < 0.05$) higher than in the control group. The immune-related hematological parameters of mouse blood showed that chronic caffeine exposure caused monocytosis, neutrophilia and eosinophilia. In group 3 we observed a 2.5 times higher ($p < < 0.05$) concentration of monocytes, in groups 3, 4 and 5 – a 2.5–2.7 times higher ($p < 0.05$) concentration of neutrophils in comparison with the control group. In the control group and group 2, the concentration of eosinophils was within the normal range. However, in groups 3, 4 and 5 higher doses of caffeine significantly (3.5, 3.6 and 4.6 times ($p < 0.05$), respectively) increased the concentration of eosinophils *versus* the control group. The mean concentration of lymphocytes and basophiles was similar in animals of all the five groups.

Our study showed that certain doses of caffeine increase the spleen index and the concentration of some immunocompetent cells. Thus, caffeine plays an important role in the development of immune resistance.

Key words: caffeine, blood cells, spleen, mice

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a wide-range most common psychoactive substance consumed on a worldwide basis. Consumption of caffeine occurs in a variety of forms, such as drinking coffee, tea, maté, or soft drinks, chewing cola nuts, consuming cocoa products or taking over-the-counter pain or slimming medications. The mean daily caffeine consumption for all adult consumers and from all sources reaches 2.4–4.0 mg/kg for a 60–70 kg subject in the United States, UK and Canada as well as 7.0 mg/kg in Scandinavia (1, 2).

Caffeine at submillimolar concentrations exerts a wide variety of physiological effects on different organisms from bacteria to humans (3). It has a wide range of effects on cardiovascular activity, including vasoconstriction, total peripheral resistance, blood flow, and so forth (4). Caffeine produces acute elevations in both systolic and diastolic blood pressure in most individuals, has analgesic properties, enhances lipolysis and fat oxidation, reduces glycogen breakdown (5, 6), increases lysozyme concentration in blood of mice (7), inhibits carcinogenesis (8); however, as a complex-forming agent it decreases the effective concentration of antitumor antibiotic actinomycin D (9, 10). Caffeine consumption is associated with a modest, but statistically significant decrease in fertility (11). Furthermore, caffeine influences many pathways involved in the cellular response to DNA damage, reducing the cell cycle delay caused by DNA damage and inhibiting repair of the damage (12); caffeine concentration in plasma might proffer a marked increase in erythrocyte sickling *in vivo* and consequently slow recovery from a sickling crisis (13). Caffeine is frequently used as a component of anti-rheumatic, anti-inflammatory and anti-pyretic drugs (14).

Correspondence to: A. Ramanavičius, Sector of Immunoanalysis and Informatics, Institute of Immunology of Vilnius University, Molėtų pl. 29, LT-2021 Vilnius, Lithuania. E-mail: almyra@imi.lt

The immune system is strongly affected by a variety of physical and psychological stressors (15). Caffeine is the most widely used psychoactive substance in the world, and it is a potential stimulant of the central nervous system (16). Mild positive subjective effects occur at low to moderate doses of caffeine (50–300 mg, *i.e.* 1–3 cups of coffee) and are described as feelings of well-being, alertness, energy and ability to concentrate. At high doses (300–800 mg), rather negative feelings such as anxiety, nervousness, and insomnia are reported, especially in volunteers who are usually caffeine-abstinents (2). The possible physical dependence on caffeine has been considered for about a decade (17–20).

In the previous study, we investigated the effect of different concentrations (2–200 mg/kg) of caffeine on mouse serum lysozyme activity and observed that its activity increased until 40 mg caffeine/kg of mouse (7). Our investigations allow to suggest that caffeine reinforces the first-line host defense against bacterial invaders. The aim of the current study was to determine the potential influence of caffeine on some immune parameters (spleen index, concentration of lymphocytes, basophiles, monocytes, neutrophils, and eosinophils), which play an important role in the development of immune resistance of the organism against infection agents.

MATERIALS AND METHODS

Reagents. EDTA and caffeine were purchased from Sigma (St. Louis, USA). All solutions were prepared by using water purified with a Millipore S.A.water purification system (Molsheim, France).

Equipment. The internal organs of mouse were weighed with a Scalte SBA33 balance (Heligenstadt, Germany). Measurements of hematological parameters in mouse blood were performed using a multiparameter, automated hematology analyzer (Hemavet Mascot from Intelimetric Ltd Oxford, USA).

Animals and housing. BALB/c mice obtained from the vivarium of the Institute of Immunology (Vilnius, Lithuania), weighing 27–28 g were used in this study. Twenty male mice were randomly allocated to treatment groups. The mice were distributed in five groups of four, housed in solid-bottomed cages with wood shavings bedding and were allowed food and water *ad libitum*. The room temperature was maintained at 21–24 °C and a 12 h light/dark cycle was employed. Approval of the Lithuanian Ethic Committee for Laboratory Animal Use was obtained prior to commencement of the experiments (N–0034).

Procedure. The mice were given 0.3 ml dose of 2 (group 2), 20 (group 3), 40 (group 4), 200 mg/kg (group 5) caffeine solution in water by oral intubation. The control group (group 1) of four mice was given water. The caffeine solutions were given at 3-day intervals during 30 days. On day 30 the mice were weighed and killed by cervical dislocation. The spleen was excised from each mouse and weighed. The spleen index (SI) was calculated as (organ weight/body weight) × 1000. Blood samples from the heart were collected by syringes, 50 µl of Na-EDTA (6%) was added into each 1 ml blood sample. The automated hematology analyzer counted neutrophils, lymphocytes, monocytes and eosinophils in the collected blood samples.

Statistical analysis. The data obtained in all test groups were compared with those obtained in the control group. Student's test (demo version) was used to determine the significance ($p < 0.05$) of the results.

RESULTS AND DISCUSSION

Our results illustrate that the mean of spleen weights was significantly higher (1.9 time, $p < 0.05$) in group 3 where animals were given 20 mg/kg of caffeine, but in group 2 and 5 the mean of spleen weight was almost the same as in the control group. The similar tendency was observed during estimation of spleen indices (SI) in the same groups of animals. The SI of group 3 was significantly higher (2.0 times, $p < 0.05$) than that of the control and other groups (Fig. 1), showing that treatment of mice with 20 mg/kg caffeine exerted a stronger influence on the weight of this organ. However, with increasing the dose of caffeine the SI had a tendency to decrease. In group 5 (200 mg/kg of caffeine) the SI was 1.4 times lower than in the control group. The spleen is a secondary lymphoid organ, where B and T lymphocytes constitute the major cellular components. The spleen enlargement can be caused by a more intensive production of lymphocytes and mo-

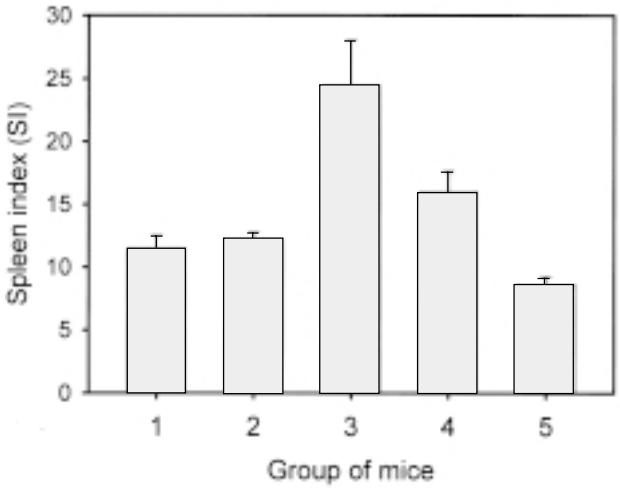

Fig. 1. Changes of spleen index depending on caffeine dose. Group 1 (control) was given doses of water, group 2 – 2 mg /kg, group 3 – 20 mg/kg ($p < 0.05$), group 4 – 40 mg/kg ($p < 0.05$), group 5– 200 mg caffeine / kg of mouse weight ($p < 0.05$)

nocytes or can be related with enhanced levels of macrophages and erythrocytes in the spleen (21).

Measurements of immune-related hematological parameters in mouse blood show that chronic caffeine exposure causes monocytosis, neutrophilia and eosinophilia, which depend on the dose of caffeine. In group 3 we observed a 2.5 times and in group 4 a 1.7 times higher ($p < 0.05$) concentration of monocytes in comparison with the control group (Fig. 2). These results explain the increased weight of spleen in these groups of mice. Monocytes circulate in the blood stream and then migrate into the tissues and differentiate into specific tissue macrophages which are actively phagocytic cells capable of ingesting and digesting exogenous antigens such as the whole bacterial cells, virus particles, and injured or dead host cells (22).

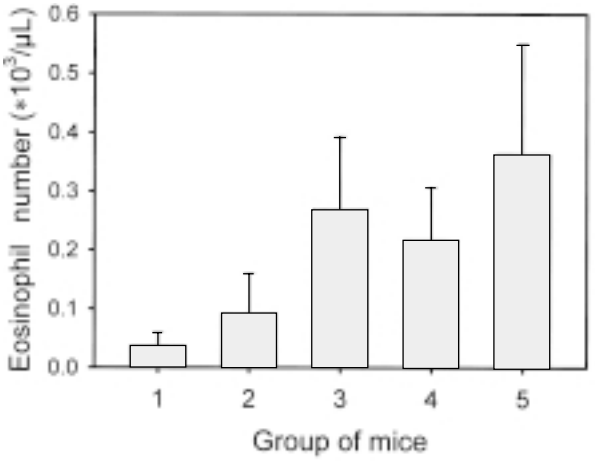

Fig. 3. Eosinophil concentration in blood of mice treated with different caffeine doses (in groups 3, 4, 5 – $p < 0.05$)

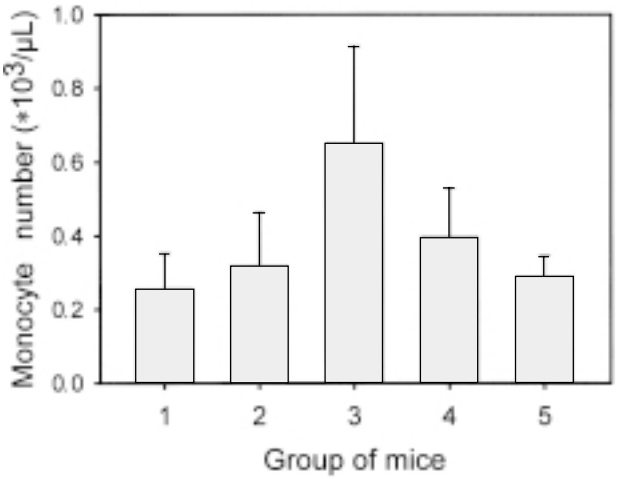

Fig. 2. Monocyte concentration in the blood of mice treated with different caffeine doses (in group 3 – $p < 0.05$)

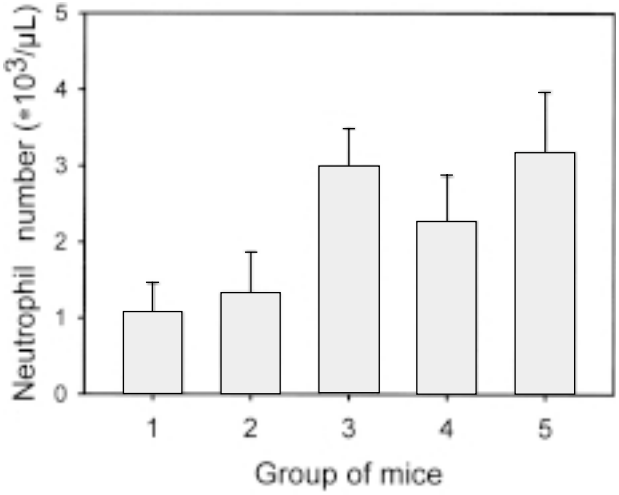

Fig. 4. Neutrophil concentration in blood of mice treated with different caffeine doses (in groups 3, 4, 5 – $p < 0.05$)

The mean concentrations of lymphocytes and basophiles were similar in animals of all five groups. The mean concentrations of erythrocytes and the concentration of hemoglobin in all mouse groups treated with different caffeine doses were approximately 1.3 times higher than in the control group.

In the control group and group 2 (treated with 2 mg/kg of caffeine) the concentration of eosinophils was within the normal range. However, with increasing the dose of caffeine the departure from the control group increased, too (Fig. 3). In group 3 treated with 20 mg/kg caffeine, departure from the control group was 3.5, while in the group treated with 40 mg/kg – 3.6, 200 mg/kg – 4.6 times higher ($p < 0.05$). A very similar tendency of caffeine influence on the concentration of neutrophils was observed (Fig. 4). In groups 3, 4, 5 treated with 20, 40, 200 mg/kg caffeine, the concentration of neutrophils increased 2.5–2.7 times ($p < 0.05$) in comparison with the control group. An increased concentration of eosinophils was observed in response to a high dose of caffeine. Caffeine can be a potential allergen (23). On the other hand, caffeine can increase migration of eosinophils and neutrophils from bone marrow to the blood stream. Eosinophils, like neutrophils, are motile, phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that their major role consists in defense against parasitic organisms. However, neutrophils are the first cells that arrive at a site of inflammation during response to many types of infection. We suggest that under the effect of caffeine bone marrow releases a higher than usual concentration of neutrophils and eosinophils, and their circulation in the blood of caffeine-treated mice shows that the organism is actively preparing for defense against infection. Thus, these results allow to suggest that caffeine enhances the immune resistance of the organism to infection.

Our study shows that certain doses of caffeine increase the spleen index and the concentration of some immunocompetent cells (monocytes, eosinophils and neutrophils), while the concentrations of lymphocytes and basophiles were similar in animals of all five groups. These results show that caffeine plays an important role in the development of immune resistance. Thus, the results presented in this study allow us to suggest that consumption of caffeine can reinforce the efficiency of anti-infection or anti-inflammatory drugs.

Received 3 February 2003
Accepted 24 march 2003

References

1. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol 1996; 34: 119–26.
2. James JE. Caffeine and Health. London: Academic Press 1991.
3. Garattini S (ed). Caffeine, Coffee, and Halth. New York: Raven Press Ltd. 1993.
4. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999; 51: 83–133.
5. Costill DL, Dalsky GP, Fink WJ. Effects of caffeine ingestion on metabolism and exercise performance. Med Sci Sports 1978; 10: 155–8.
6. Pasman WJ, van Baak MA, Jeukendrup AE, de Haan A. The effect of different dosages of caffeine on endurance performance time. Int J Sports Med 1995; 16: 225–30.
7. Ramanavičienė A, Žukienė V, Ačaitė J, Ramanavičius A. Influence of caffeine on lysozyme activity in the blood serum of mice. Acta Medica Lituanica 2002; 4: 241–4.
8. Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS et al. Topical applications of caffeine or (–)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. Proc Natl Acad Sci USA 2002; 99: 12455–60.
9. Nurminen ML, Niittynen L, Korpela R, Vapaatalo H. Coffee, caffeine and blood pressure: A critical review. Eur J Clin Nutr 1999; 53: 831–9.
10. Sawynok J, Yakahi TL. Caffeine as an analgesic adjuvant: A review of pharmacology and mechanisms of action. Pharmacol Rev 1993; 45: 43–85.
11. Barbieri RL. The initial fertility consultation: Recommendations concerning cigarette smoking, body massindex, and alcohol and caffeine consumption. Am J Obstet Gynecol 2001; 185: 1168–73.
12. Murnane JP. Cell cycle delay in response to DNA damage in mammalian cells: a historical perspective. Cancer Metast Rev 1995; 14: 17–29.
13. Uwakwe AA, Onwuegbuke C, Nwinuka NM. Effect of caffeine on the polymerization of HbS and sickling rate/osmotic fragility of HbS erythrocytes. J Appl Sci Envir Man 2002; 6: 69–72.
14. Nogowska M, Muszalska I, Zając M. Simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine in pharmaceutical preparations. Chem Anal (Warsaw) 1999; 44: 1041–8.
15. Ottaviani E, Franceschi C. The neuroimmunology of stress from invertebrates to man. Progress Neurobiol 1996; 48: 421–40.
16. Gilbert RM. Caffeine consumption. In: Spiller GA, ed. The methylxanthine beverages and foods: chemistry consumption, and health effects. New York; Liss 1984: 185–213.
17. Griffiths RR, Woodson PP. Caffeine physical dependence, a review of human and laboratory animal studies. Psychopharmacology 1988; 94: 437–51.
18. Griffiths RR, Evans SM, Heishman SJ, Preston KL, Sannerud CA, Wolf B, Woodson PP. Low-dose caffeine physical dependence in humans. J Pharmacol Exp Ther 1990; 255: 1123–32.
19. Holtzman SG. Caffeine as a model drug of abuse. Trends Pharmacol Sci 1990; 11: 355–6.
20. Strain EC, Mumford GK, Silverman K, Griffiths RR. Caffeine dependence syndrome. Evidence from case histories and experimental evaluations. JAMA 1994; 172: 1043–8.
21. Pieškus J. Veterinarinės imunologijos pagrindai. Kaunas: Morkūnas ir Ko 2001.
22. Kuby J. Immunology. New York: W. H. Freeman and Company 1997.
23. Jurgelionienė S, Šergalienė O. Klinikinių laboratorinių tyrimų metodai. Vilnius: Mokslo ir enciklopedijų leidykla 1995.

A. Ramanavičienė, J. Ačaitė, A. Dringelienė, A. Markevičius, A. Ramanavičius

KOFEINO POVEIKIS PELIŲ IMUNOKOMPETENTINĖMS LĄSTELĖMS

S a n t r a u k a

Šio darbo tikslas buvo ištirti kofeino poveikį kai kuriems pelių imunologiniams rodikliams (blužnies indeksui, limfocitų, bazofilų, monocitų, neutrofilų ir eozinofilų skaičiui). Tyrimo metu pelės kas 3 dienos 30 dienų buvo girdomos skirtingos koncentracijos vandeniniu kofeino tirpalu (0,3 ml): I grupė (kontrolė) buvo girdoma vandeniu, II grupė – 2, III grupė – 20, IV grupė – 40, V grupė – 200 mg kofeino/kg svorio. Nustatėme, kad III grupės pelių gerokai padidėjo blužnis, o blužnies indeksas buvo du kartus ($p < 0,05$) didesnis nei kontrolinių pelių. Be to, monocitų koncentracija III grupėje 2,5 karto ($p < 0,05$), neutrofilų koncentracija III–V grupėje 2,5–2,7 karto ($p < 0,05$) buvo didesnė lyginant su atitinkamais kontrolinės grupės rodikliais. Didinant kofeino dozę, didėja ir eozinofilų koncentracija: III grupėje – 3,5 karto, IV grupėje – 3,6 karto, V grupėje – 4,6 karto ($p < 0,05$) lyginant su kontroline grupe.

Šio darbo rezultatai rodo, kad tam tikros kofeino koncentracijos didina blužnies indeksą ir kai kurių imunokompetentinių ląstelių (monocitų, eozinofilų, neutrofilų) koncentraciją kraujyje. Taigi galima teigti, kad kofeinas stiprina organizmo atsparumą, aktyvina organizmo imuninę būklę.

Raktažodžiai: kofeinas, kraujo ląstelės, blužnis, pelės