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Effect of stress-induced lipid peroxidation on functions of rat peritoneal macrophages

V. Nimet İzgüt-Uysal[a,*], Ruken Tan[a], Mehmet Bülbül[a], Narin Derin[b]

[a] *Akdeniz University, Faculty of Medicine, Department of Physiology, 07070 Antalya, Turkey*
[b] *Akdeniz University, Faculty of Medicine, Department of Biophysics, 07070 Antalya, Turkey*

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Abstract

The aim of the present study was to investigate the effects of stress-induced lipid peroxidation on macrophages' functions. Animals were subjected to 4 h immobilization at 4 °C in restraining devices. The peritoneal macrophages obtained from rats exposed to cold and restraint stress exhibited an increase in lipid peroxidation and a decline of chemotaxis and phagocytosis compared with control rats. After supplementation with vitamin E, the increment in thiobarbituric acid reactive substances (TBARS) content as the oxidative stress marker and the decline of chemotaxis and phagocytosis in peritoneal macrophages observed during cold-restraint stress was significantly removed. No significant change in catalase activity of peritoneal macrophages was observed in groups exposed to cold-restraint stress and treated with vitamin E. These findings indicate that phagocytic and chemotactic capacities of peritoneal macrophages are decreased by cold-restraint stress and this effect of stress may be related to lipid peroxidation.

Keywords: Lipid peroxidation; Phagocytosis; Chemotaxis; Catalase activity; Vitamin E

1. Introduction

Stress is accepted as a response of an organism to external stimuli or changes (Dorshkind and Horseman, 2001; Peng et al., 2000). Numerous studies show that stress can be immunosuppressive. In these studies, stress has also been shown to suppress different immune parameters, e.g. delayed type hypersensitivity, antibody production, NK activity, leukocyte proliferation, skin homograft rejection, and virus-specific T cell activity (Dorshkind and Horseman, 2001; De Castro et al., 2000; Dhabhar, 2000).

It is well known that stress induces formation of reactive oxygen species (ROS) and leads to the oxidative injury in various tissues (De Castro et al., 2000; Nishida et al., 1997; Toleikis and Godin, 1995). Oishi and Machida (2002) have shown that a significant increase in plasma TBARS was observed during and after the stress. The activation of immune cells can be a source of the stress-induced ROS production and antioxidant enzymes in immune cells play an important role in preventing the ROS-induced injury (De Castro et al., 2000; Babior, 2000; Victor et al., 2003). Oxidant–antioxidant balance is critical for immune cell functions because of its protective effect of the maintenance of cell membrane integrity and functionality (Pawlak et al., 1998a, 1998b; Knight, 2000; Celada and Nathan, 1994).

Immune cells are particularly sensitive to oxidative stress because of the presence of polyunsaturated fatty acids in their plasma membranes and production of ROS, which is part of their normal function (Pawlak et al., 1998a; Knight, 2000; Celada and Nathan, 1994). Moreover, membrane-related functions are critical in maintaining normal function of immune cells and their ability to defend against foreign antigens (Biselli et al., 1996; Yuli et al., 1982). These funtions are highly sensitive to ROS (Babior, 2000; Victor et al., 2003).

Macrophages can survive exposure to endogenously generated ROS for a long period and play important

* Corresponding author. Tel.: +090-242-2274483; fax: +090-242-2274483.
E-mail address: nimetu@akdeniz.edu.tr (V. Nimet İzgüt-Uysal).

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roles in phagocytosis-mediated host defence against microbial infection. The role of superoxide anion liberation by the macrophages in the defence against pathologic agents is well known (De Castro et al., 2000; Babior, 2000; Alvarez et al., 1996). However, studies are still required concerning the effect of liberation of oxidizing agents by peritoneal macrophages, and their effects on changes due to stress. The present work was aimed at examining the effect of cold and restraint stress-induced lipid peroxidation on macrophages' functions.

2. Materials and methods

2.1. Animals

Thirty-two male Wistar rats (200–220 g) were used. The animals were fed with a standard diet and water ad libitum and divided into three groups (control group, $n = 12$ and stress group, $n = 10$, vitamin E group, $n = 10$). The stress group was exposed to immobilization and cold stress for 4 h at 4 °C before the experimental protocol. The vitamin E group was exposed to equivalent stress and treated with vitamin E. Vitamin E was injected intramuscularly in only one dose of 30 mg/kg, 5 min before stress was applied. Each group was housed at room temperature (22 ± 1 °C) and 12 h day–night cycle. The study protocol was approved by the Akdeniz University Animal Care and Users Committee.

2.2. Preparation of peritoneal macrophages

The animals were anesthetized with diethyl ether. Ten milliliters of Krebs-phosphate buffer solution was injected intraperitoneally and after 3 min, their abdomens were opened by a midline incision without haemorrhage and intraperitoneal fluid collected. The cells were washed, counted and added to RPMI-1640 medium at a final concentration of 2×10^6 viable cells per milliliters. Cell viability was >95%, as determined by the trypan blue exclusion dye test.

2.3. Determination of TBARS

Lipid peroxidation was measured by the method of Stocks and Offerman (1972). Samples were adjusted by dilution with the homogenization buffer solution to 2×10^6 macrophages per milliliters. Two milliliters of trichloroacetic acid (TCA) was added into each tube containing 3 ml sample and homogenized for 15 s by vortex. Tubes were centrifuged at 4000 rpm for 10 min (Heraeus, Labofuge 200). Three milliliters of the clear supernatant was added to the tubes, as was 1 ml thiobarbituric acid (TBA) and the tubes were kept in the boiling water for 15 min. Cooled samples were assayed by spectrophotometry at 532 nm (Shimadzu UV-1601). The reagent 1,1,3,3-tetraethoxypropane was used as a standard. The results were calculated as nmol TBA per gram protein. Protein was determined by the method of Lowry.

2.4. Measurement of catalase activity

Intracellular catalase activity was measured according to the method described by Aebi (1987). To measure the intracellular catalase of 2×10^6 macrophages in 0.5 ml, cell lysates were prepared with a specific lysis buffer (10 mmol/l EDTA, 2% Triton-X, 0.05% deoxycholic acid in phosphate buffer saline, pH 7.4) and diluted with 50 mmol/l phosphate buffer (pH 7) and centrifuged at 3000 rpm for 10 min at 4 °C (Heraeus, Biofuge 15R). Two milliliters of sample was dispensed into a quartz cuvettte, followed by 1 ml of 30 mmol/l H_2O_2 in phosphate buffer (pH 7.0). Catalase activity was measured by the consumption of H_2O_2 at 240 nm in spectrophotometer (Shimadzu UV-1601). One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μmol/H_2O_2/min at 25 °C and pH 7.0. Results are expressed as units of catalase activity/g protein.

2.5. Measurement of chemotactic activity

Preparation of zymosan-activated serum (ZAS, 10%) was by the method of Goldstein et al. (1975), involving incubation of rat serum with boiled and washed zymosan particles at 1 mg/ml for 45 min at 37 °C. The zymosan particles were removed by centrifugation (1000 g for 10 min) before the supernatant was collected and stored at −20 °C in small aliquots until used. For chemotactic activity, the ZAS was diluted 1:10 with incubation medium.

The chemotaxis assay was performed by Boyden's method (1962), using a nitrocellulose filter of 8 μm pore size (Schleicher and Schuell AE 99 Membrane Filter). A sample of 0.5 ml was prepared to contain 2×10^6 macrophages per ml with RPMI-1640 medium and added to the upper compartment of the Boyden chamber. A 0.5 ml ZAS was injected to the lower compartment of the chamber and incubated for 45 min at 37 °C. After the incubation period, membrane filter was removed and the cells were stained with hematoxylin. The distance in micrometers migrated by macrophages to the lower face of the filter was determined under a light microscope (×1000; Olympus B201). Data were expressed as the average of 3 randomly chosen fields.

2.6. Determination of phagocytic activity

One hundred microliters of 1% activated charcoal was added to 100 μl of cell suspension and incubated for 1 h at 37 °C. After the incubation, number of the

phagocyted charcoal particles by the macrophages was counted and an average of 100 cells were used to determine the phagocytic activity.

2.7. Statistics

Data are presented as the means ± SE. Statistical analyses were performed using a Mann–Whitney test and p values of <0.05 were considered significant.

3. Results

3.1. TBARS content of macrophages

The changes observed in lipid peroxidation status in peritoneal macrophages are shown in Fig. 1. Cold-restraint stress increased TBARS content of peritoneal macrophages as an index of lipid peroxidation with statistical significance when compared to control rats (196.07 ± 56.14 nmol/g protein in control group, 278.1 ± 53.8 nmol/g protein in stress group). Vitamin E treatment of stressed rats significantly prevented the increase in lipid peroxidation (201.0 ± 33.3 nmol/g protein, $p < 0.05$ when compared to stress group).

3.2. Catalase activity

We examined the levels of intracellular catalase activity that catalyze H_2O_2 to H_2O in peritoneal macrophages. There was no significant difference in catalase activity of peritoneal macrophages between the groups (65.4 ± 16.7 U/g protein in control group, 91.2 ± 28.4 U/g protein in stress group, 78.6 ± 19.2 U/g protein in stressed rats treated with vitamin E).

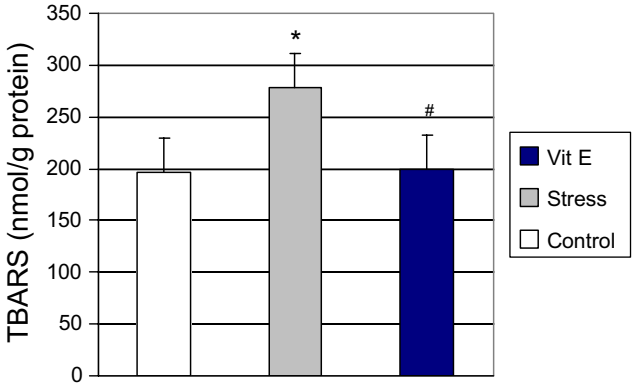

Fig. 1. Effects of cold-restraint stress and vitamin E treatment on TBARS production of peritoneal macrophages. Data represent mean ± SE, $*p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. stress group.

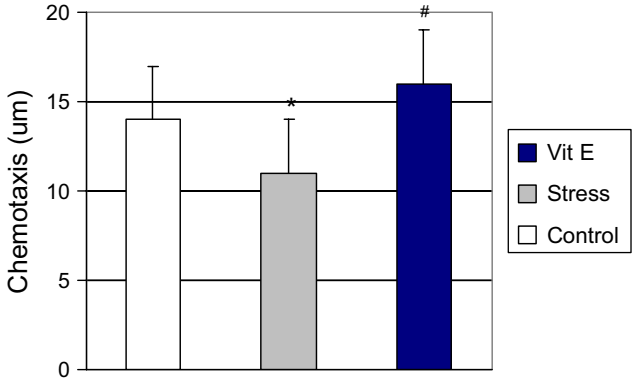

Fig. 2. Effects of cold-restraint stress and vitamin E treatment on chemotactic activity of peritoneal macrophages. Data represent mean ± SE, $*p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. stress group.

3.3. Chemotactic activity

Macrophages obtained from stressed group presented lower chemotactic capacity than that of control group with statistical significance (14.6 ± 3.2 μm in control group and 11.1 ± 1.7 μm in stress group). Antioxidant supplementation with vitamin E prevented the decline of chemotaxis due to cold-restraint stress (16.0 ± 3.2 μm, $p < 0.05$ when compared with stress group) (Fig. 2).

3.4. Phagocytic activity

Fig. 3 shows the phagocytic activity of peritoneal macrophages. The number of the particles phagocyted by peritoneal macrophages from stress group was significantly ($p < 0.05$) lower when compared to that of the control animals (6.2 ± 0.8 particles in control group and 4.6 ± 0.8 particles in stress group). The decline in phagocytosis due to stress administration disappeared with vitamin E treatment (7.4 ± 1.0 particles in vitamin E treated stress group).

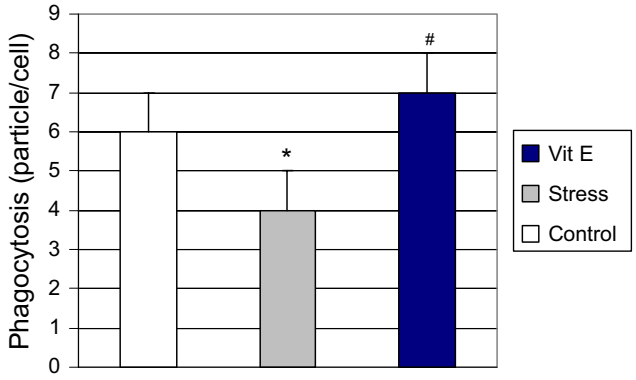

Fig. 3. Effects of cold-restraint stress and vitamin E treatment on phagocytic activity of peritoneal macrophages. Data represent mean ± S.E., $*p < 0.05$ vs. control group, $^{\#}p < 0.05$ vs. stress group.

4. Discussion

This study was designed to investigate the effect of cold-restraint stress-induced lipid peroxidation on the functions of peritoneal macrophages. For the supplementation of antioxidant activity, vitamin E was used and TBARS as the oxidative stress marker, and catalase activity were investigated. Numerous previous studies (Dorshkind and Horseman, 2001; De Castro et al., 2000; Dhabhar, 2000; Vizi, 1998) along with our data support the hypothesis that stress situations are associated with altered immune functions. We showed that peritoneal macrophages are not resistant to oxidative stress. Cold-restraint stress was found to induce oxidative stress through decreased chemotactic and phagocytic activities, and increased lipid peroxidation, as shown by enhanced TBARS levels.

Macrophages can survive for a long period to an exposure of exogenously generated reactive oxygen species and play important roles in phagocytosis-mediated defence against microbial infection (De Castro et al., 2000; Babior, 2000; Alvarez et al., 1996). Phagocytosis stimulates respiratory burst, which contains several chemical events including ROS production (De Castro et al., 2000; Babior, 2000; Pawlak et al., 1998a). ROS can function as signaling molecules in regulation of fundamental cell activities such as cell growth and cell adaptation responses, whereas at higher concentrations, ROS can cause cellular injury and death (Lum and Roebuck, 2001). Excess ROS generated by phagocytes may damage biologically important macromolecules. Membrane lipids are particularly vulnerable to peroxidation. Lipid peroxidation damages the cell membrane, with the result that an altered membrane fluidity and cell membrane-related function occur, including chemotaxis and phagocytosis (Peng et al., 2000; Babior, 2000; Victor et al., 2003; Pawlak et al., 1998a, 1998b; Knight, 2000). Immune cell functions are specially linked to reactive oxygen species generation, the oxidant–antioxidant balance is essential for these cells. Intake of antioxidant vitamins resulted in a significant increase in the phagocytic function of polymorphonuclear neutrophils as well as in a significant decrease of lipid peroxides (Del Rio et al., 1998; De La Fuente et al., 2002). It was found that vitamin E possess a high immunomodulating and antioxidant activity under the acute cold-induced stress conditions. Vitamin E is an efficient antioxidant that functions as a ''chain breaker'' in lipid peroxidation of cell membranes (Uteshev et al., 2001). It is demonstrated that restraint stress induced a decrease in the level of activities of antioxidant enzymes, while the levels of TBARS were found elevated. Antioxidant, especially vitamin E, supplementation enhances cell-mediated immunity (Zaidi and Banu, 2004).

Peritoneal macrophages are sensitive to exogenous H_2O_2 (Kamuro et al., 2001). In accordance with the susceptibility to H_2O_2, peritoneal macrophages express lower activity of catalase and an inadequacy to induce catalase gene expression in response to H_2O_2. Peritoneal macrophages produce and release a large amount of H_2O_2 because of their low catalase activity. Kamuro et al. (2001) demonstrated that macrophages (except peritoneal macrophages) have a marked ability to induce catalase gene expression by exposure of H_2O_2. We measured catalase activity of peritoneal macrophages because peritoneal macrophages are not resistant to H_2O_2. In agreement with findings of Kamuro et al., catalase activity in peritoneal macrophages isolated from stressed rats was found to be higher than that of control rats, but not significant statistically in present study.

Phagocytic cells (neutrophils, monocytes, and macrophages) are the first line of defence system of the organism against the infectious agents (Babior, 2000; Alvarez et al., 1996). Macrophages accomplish nonspecific immune function through what is known as phagocytosis. Phagocytic function can be divided into several stages as chemotaxis, ingestion and killing of infectious agents by producing superoxide anion radicals (Babior, 1992; Witko-Sarsat et al., 2000). According to our data, exposure to cold-restraint stress rendered peritoneal macrophages incapable of chemotactic and phagocytic activities. Our findings are also corroborated by demonstration of Victor et al. (2003) that the chemotactic activity in peritoneal macrophages from stressed rats is reduced. The decline in macrophages' functions in stress conditions in our study may be related to the increase of lipid peroxidation in macrophages, which is because vitamin E treatment was found to be effective in preventing the stress-induced decrease of phagocytosis, chemotaxis activities and increase of TBARS levels in this study. The findings of this study indicate that the increase in lipid peroxidation causes the inhibition of chemotaxis and phagocytosis of macrophages.

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References

Aebi H. Catalase of enzymatic analysis. In: Bergmeyer HU, editor. Enzyme 1: oxyreductases, transferases, vol. 3. Weinheim: VCH Verlagsgesellschaft; 1987. p. 273–85.

Alvarez E, Machado A, Sobrino F, Maria S. Nitric oxide and superoxide anion production decrease with age in resident and activated rat peritoneal macrophages. Cell Immunol 1996;169:152–5.

Babior BM. Neutrophil function as related to neutrophil–endothelial cell interactions. Nouv Rev Fr Hematol 1992;34(Suppl):S29–35.

Babior BM. Phagocytes and oxidative stress. Am J Med 2000;109(1):33–44.

Biselli R, Farrace S, De Simone C, Fattorossi A. Potentiation of human polymorphonuclear leukocyte activation by atrial natriuretic peptide. Inhibitory effect of carnitine congeners. Inflammation 1996;20(1):33–42.

Boyden SV. The chemotactile effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J Exp Med 1962;115:453.

Celada A, Nathan C. Macrophage activation revisited. Immunol Today 1994;15:100–2.

De Castro CMMB, De Castro RM, De Medeiros AF, Santos AQ, Ferreira E Silva, De Lima Filho JS. Effect of stress on the production of O_2^- in alveolar macrophages. J Neuroimmunol 2000; 108(1–2):68–72.

De La Fuente M, Miquel J, Catalan MP, Victor VM, Guayerbas N. The amount of thiolic antioxidant ingestion needed to improve several immune functions is higher in aged than in adult mice. Free Radic Res 2002;36(2):119–26.

Del Rio M, Ruedas G, Medina S, Victor VM, De la Fuente M. Improvement by several antioxidants of macrophage function in vitro. Life Sci 1998;63(10):871–81.

Dhabhar FS. Acute stress enhances while chronic stress suppresses skin immunity. The role of stress hormones and leukocyte trafficking. Ann N Y Acad Sci 2000;917:876–93.

Dorshkind K, Horseman ND. Anterior pituitary hormones, stress, and immune system homeostasis. BioEssays 2001;23:288–94.

Goldstein IM, Roos D, Kaplan HB, Weismann G. Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytes. J Clin Invest 1975;56: 1155–63.

Kamuro I, Keicho N, Iwamoto A, Akagawa KS. Human alveolar macrophages and granulocyte-macrophage colony-stimulating factor-induced monocyte derived macrophages are resistant to H_2O_2 via their high basal and inducible levels of catalase activity. J Biol Chem 2001;276(26):24360–4.

Knight JA. Review: free radicals, antioxidants and the immune system. Ann Clin Lab Sci 2000;30(2):145–58.

Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol 2001;280:C719–41.

Nishida K, Ohta Y, Kobayashi T, Ishuguro I. Involvement of the xanthine–xanthine oxidase system and neutrophils in the development of acute gastric mucosal lesions in rats with water immersion restraint stress. Digestion 1997;58(4):340–51.

Oishi K, Machida K. Different effects of immobilization stress on the mRNA expression of antioxidant enzymes in rat peripheral organs. Scand J Clin Lab Invest 2002;62(2):115–21.

Pawlak W, Kedziora J, Zolynski K, Kedziora-Kornatowska K, Blaszczyk J, Witkowski P. Free radicals generation by granulocytes from men during bed rest. J Gravity Physiol 1998a;5(1):P131–2.

Pawlak W, Kedziora J, Zolynski K, Kedziora-Kornatowska K, Blaszczyk J, Witkowski P, et al. Effect of long term bed rest in men on enzymatic antioxidative defence and lipid peroxidation in erythrocytes. J Gravit Physiol 1998b;5(1):163–4.

Peng J, Jones GL, Watson K. Stress proteins as biomarkers of oxidative stress: effects of antioxidant supplements. Free Radic Biol Med 2000;28(11):1598–606.

Stocks J, Offerman E. The susceptibility to autoxidation of human red cell lipids in health and disease. Br J Haematol 1972;23:713–24.

Toleikis PM, Godin DV. Alteration of antioxidant status in diabetic rats by chronic exposure to physiological stressors. Pharmacol Biochem Behav 1995;52(2):355–66.

Uteshev BS, Bystrova NA, Brovkina IL, Avakian AR. Immunomodulating and antioxidant effects of vitamins A and E during air and immersion cooling. Eksp Klin Farmakol 2001;64(1):60–2.

Victor VM, Rocha M, De la Fuente M. Regulation of macrophage function by the antioxidant N-acetylcysteine in mouse-oxidative stress by endotoxin. Int Immunopharmacol 2003;3(1):97–106.

Vizi ES. Receptor mediated local fine-tuning by noradrenergic innervation of neuroendocrine and immune systems. Stress of life: from molecules to man. Ann N Y Acad Sci 1998;30:388–96.

Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest 2000;80(5):617–53.

Yuli I, Tamonaga A, Synderman R. Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. Proc Natl Acad Sci U S A 1982;79: 5906–10.

Zaidi SM, Banu N. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. Clin Chim Acta 2004; 340(1–2):229–33.