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Colchicine Modulates Oxidative Stress in Serum and Leucocytes from Remission Patients with Family Mediterranean Fever Through Regulation of Ca^{2+} Release and the Antioxidant System

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Abstract We investigated the effects of colchicine on oxidative stress and Ca^{2+} release in serum and polymorphonuclear leucocytes (PMNs) of Familial Mediterranean Fever (FMF) patients with attack, remission and unremission periods. Eighteen FMF patients and six age-matched healthy subjects in four groups were used. The first group was a control. The second group included patients with active FMF. The third and fourth groups were patients with remission and unremission, respectively. Colchicine (1.5 mg/day) was given to the third and fourth groups for 1 month. PMN cells, serum lipid peroxidation and intracellular Ca^{2+}-release levels in the attack and unremission groups were higher than in those in controls, although they were lower in the remission group than in the attack group. Serum vitamin E and β-carotene concentrations were higher in the remission group than in the control and attack groups. However, PMN, serum lipid peroxidation and Ca^{2+}-release levels were further increased in the unremission group compared to the attack group. Glutathione peroxidase, reduced glutathione and vitamin A values in the four groups did not change by FMF and colchicine. In conclusion, we observed that colchicine induced protective effects on oxidative stress by modulating vitamin E, β-carotene and Ca^{2+}-release levels in FMF patients with a remission period.

Keywords Familial Mediterranean Fever · Inflammation · Ca^{2+} release · Oxidative stress · Neutrophil

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Introduction

Reactive oxygen species (ROS) and lipid peroxides are produced by a free radical chain reaction, which can also be initiated by ROS (Halliwell 2006). ROS contribute to cell damage (Nazıroğlu 2009). ROS also cause injury by reacting with biomolecules such as lipids, proteins and nucleic acids as well as by depleting enzymatic and/or nonenzymatic antioxidants in the leucocytes (Bréchard and Tschirhart 2008). There is also evidence that ROS play an important role in the pathogenesis of many diseases, particularly inflammatory diseases (Kamanli et al. 2004; Guz et al. 2009).

Familial Mediterranean Fever (FMF) is a recessive disorder characterized by episodes of fever with serositis or synovitis. It particularly affects Jewish, Armenian, Arab and Turkish communities in the Mediterranean Sea region and shows autosomal recessive inheritance. The male-to-female ratio is 1.5–2. FMF is thought to be due to a disorder in the configuration of the inflammatory response (Guz et al. 2009). The coexistence of increased oxidative stress with symptoms of FMF in patients, as evidenced by defective plasma antioxidant defenses in association with enhanced susceptibility to lipid peroxidation (LP), has been reported (Kirkali et al. 2008; Gurbuz et al. 2005). Significant correlations were also found between the severity of FMF attacks and alterations in glutathione peroxidase

(GSH-Px) activity as well as LP levels (Gurbuz et al. 2005).

Colchicine, an alkaloid traditionally extracted from *Colchicum autumnale* (meadow saffron), remains a common choice in the treatment of acute gout arthritis. Colchicine has been commonly used in the treatment of FMF (Modriansky et al. 2002). However, it has a relatively low therapeutic index in FMF because its effects are not well known in molecular pathways. In spite of its apparent affinity for a lipid environment and its effects on membranes (Mons et al. 2000), the proposed antioxidant effect of colchicine in liver cells has been discounted (Das et al. 2000; Modriansky et al. 2002). Most of colchicine's biological effects were ascribed to its tubulin-binding ability (Mons et al. 2000). Polymorphonuclear leucocyte (PMN) cells are primarily or secondarily involved in the pathogenesis of FMF (Kirkali et al. 2008). They are key players in the inflammatory process, during which they are exposed to a variety of agonists that signal mostly through heterotrimeric G protein–coupled receptors. An increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important step within the multitude of serial or parallel signaling events that participate in the activation of neutrophil reactions such as chemotaxis and release of ROS (Pantaler and Lückhoff 2009). The release of superoxide radicals by NADPH oxidase that follows stimulation of the formyl peptide receptor requires an increase in $[Ca^{2+}]_i$ (Bréchard and Tschirhart 2008). Colchicine inhibited arachidonic acid release and 5-lipoxygenase action in alveolar macrophages via regulation of Ca^{2+} influx (Peters-Golden et al. 1996). However, the mechanisms of these effects are not fully understood in PMNs.

Since the modulatory effects of colchicine on cellular survival and death in molecular pathways of patients with FMF have not been clarified, we focused on the dual effect of colchicine in human PMN cells by checking its role on LP and antioxidant levels and Ca^{2+} release from intracellular stores evoked by *N*-formyl-*L*-methionyl-*L*-Leucyl-*L*-phenylalanine (fMLP) as a calcium mobilizing agonist.

Subjects and Methods

Chemicals

All chemicals (cumene hydroperoxide, KOH, NaOH, thiobarbituric acid, 1,1,3,3-tetraethoxypropane, 5,5-dithiobis-2 nitrobenzoic acid, tris-hydroxymethyl-aminomethane, glutathione, butylhydroxytoluol, fMLP, thapsigargin, digitonin and ethylene glycol-bis[2-aminoethyl-ether]-*N,N,N′,N′*-tetraacetic acid [EGTA]) were obtained from Sigma-Aldrich (St. Louis, MO) and all organic solvents (n-hexane, ethyl alcohol) were purchased from Merck (Darmstadt, Germany). Fura-2 acetoxymethyl ester was purchased from Invitrogen (Carlsbad, CA, USA). All reagents were analytical grade. All reagents except the phosphate buffers were prepared daily and stored at +4°C. Reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagent containers were refilled. Phosphate buffers were stable at +4°C for 1 month.

Patients

The study was approved by the Ethics Committee, Medical Faculty, Suleyman Demirel University (Isparta, Turkey). All participants gave written consent, confirming their acceptance for giving blood through the vena brachialis, and were informed about all experimental procedures. Patients were diagnosed and classified by clinicians of the Internal Medicine Clinic, and the control group was selected among healthy parents or siblings.

The study was performed in 18 FMF patients (12 male, 6 female) aged 29–47 years, mean age 30.3 years (31.5 years for men, 28.6 years for women). None of them had an alcohol abuse problem. They had no proteinuria, nephritic syndrome or kidney disease. They had not received any systemic therapy which might affect cellular immunity during the 2 weeks prior to sample collection. The control group consisted of six healthy volunteers precisely matched for age and sex. The women who were included in the study had not been taking oral contraceptives for at least 6 months before sample collection. The clinical diagnosis of FMF was based on the Tel-Hashomer criteria (Livneh et al. 1997), and the median duration of illness was 8.6 years (range 1–20).

Study Groups

Six control and eighteen patients with FMF were equally divided into four groups as follows:

Control group ($n = 6$): Age-matched healthy people
Attack group ($n = 6$): FMF patients withdrawn from colchicine treatment 10 days before collection of blood samples
Remission group ($n = 6$): Patients controlled by therapeutic dose of colchicine (1.5 mg/day) for 30 days
Unremission group ($n = 6$)*:* FMF attacks not controlled by colchicines, although patients were daily taking a therapeutic dose of colchicine (1.5 mg/day) for 30 days

After an overnight fast, blood samples of the controls and patients were drawn from the antecubital vein into tubes with and without anticoagulant. Serum and neutrophil samples were obtained from the blood samples. Serum and half of the neutrophil samples were stored at −33°C and used for LP and antioxidant analysis within 1 month.

Remaining neutrophil samples were daily used for $[Ca^{2+}]_i$ analysis.

Isolation of Human Neutrophils

Neutrophils were isolated from peripheral whole blood of healthy volunteers and patients with FMF, as described previously (Pantaler and Lückhoff 2009), by centrifugation through Ficoll. Half of the cells were stored for antioxidant analyses. The remaining cells were used for measurement of $[Ca^{2+}]_i$. Sterile solutions used for neutrophil isolation were phosphate-buffered saline from GIBCO Invitrogen (Istanbul, Turkey), 6% hydroxyl ethyl starch solution in isotonic NaCl (Plasmasteril) from Fresenius (Bad Homburg, Germany) and Ficoll-Paque PLUS from GE Healthcare Bio-Sciences (Uppsala, Sweden). The loading buffer contained HEPES (20 mM), NaCl (138 mM), KCl (6 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.6 mM) and glucose (5.5 mM), pH 7.4, and was supplemented with 2% (v/v) of autologous serum. The measuring buffers did not contain serum but were otherwise identical to the loading buffer when a normal extracellular Ca^{2+} concentration was explored.

Measurement of $[Ca^{2+}]_i$

PMN cells were loaded with 4 μM Fura-2/AM in loading buffer with 5×10^6 cells/ml for 45 min at 37°C in the dark, washed twice, incubated for an additional 30 min at 37°C to complete probe de-esterification and resuspended in loading buffer at a density of 3×10^6 cells/ml according to a procedure published elsewhere (Uğuz et al. 2009; Pantaler and Lückhoff 2009). The four groups were exposed to fMLP for stimulating intracellular Ca^{2+} release. Fluorescence was recorded from 2-ml aliquots of a magnetically stirred cellular suspension at 37°C using a spectrofluorometer (Carry Eclipse; Varian, Sydney, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored using the Fura-2/AM 340/380 nm fluorescence ratio and calibrated according to the method of Grynkiewicz et al. (1985). In the experiments where calcium-free medium is indicated, Ca^{2+} was omitted and 2 mM EGTA was added.

Ca^{2+} release was estimated using the integral of the rise in $[Ca^{2+}]_i$ for 40 s after addition of fMLP (Espino et al. 2009; Uğuz et al. 2009). Ca^{2+} release is expressed in nanomoles, taking a sample every second as previously described (Heemskerk et al. 1997).

LP Determinations

LP levels in the serum and neutrophil samples were measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966). Thiobarbituric acid–reactive substances were quantified by comparing the absorption to the standard curve of malondialdehyde (MDA) equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. LP values in neutrophil and serum samples were expressed as micromoles per gram of protein and as nanomoles per milliliter, respectively.

Reduced Glutathione, GSH-Px and Protein Assay

The reduced glutathione (GSH) content of the serum and neutrophil samples was measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of serum and neutrophil samples were measured spectrophotometrically at 37°C and 412 nm according to the method of Lawrence and Burk (1976). The protein content in neutrophil samples was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Serum Vitamin A, Vitamin E and β-Carotene Analyses

Vitamins A (retinol) and E (α-tocopherol) were determined in serum samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). Serum samples (250 μl) were saponified by the addition of 0.3 ml KOH (60% and w/v in water) and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70°C for 30 min. After cooling the samples on ice, 2 ml of water and 1 ml of n-hexane were added and mixed with the samples, which were then rested for 10 min to allow phase separation. An aliquot of 0.5 ml of n-hexane extract was taken, and vitamin A concentrations were measured at 325 nm. Then, reactants were added and the absorbance value of hexane was measured in a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan) at 535 nm. Calibration was performed using standard solutions of all-*trans* retinol and α-tocopherol in hexane.

Concentrations of β-carotene in serum samples were determined according to the method of Suzuki and Katoh (1990). Two milliliters of hexane were mixed with 250-μl serum samples. The concentration of β-carotene in hexane was measured at 453 nm in a spectrophotometer.

Biochemical Analysis

Hemoglobin levels and neutrophil and leucocyte counts were measured in a cell counter (Beckman Coulter LH 70 hematology analyzer; Beckman Coulter, Brea, CA). Erythrocyte sedimentation rate (ESR) was measured by routine Westergren method. C-reactive protein (CRP) is one of the acute-phase proteins that increase during systemic inflammation, and serum CRP values in patients with

FMF were measured in a nephelometer (Delta Seac Radim, Pomezia, Italy).

Statistical Analysis

Data are expressed as means ± SEM of the numbers of determinations. Statistical significance was analyzed using the SPSS program (9.05; SPSS, Inc., Chicago, IL). To compare the different treatments, statistical significance was calculated by the Mann–Whitney *U*-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of Colchicine Treatment on Intracellular Ca^{2+} Release in Neutrophils of Patients with FMF

Effects of colchicine treatment on intracellular Ca^{2+} release in PMNs are shown in Fig. 1. Intracellular Ca^{2+} release in the cells was significantly ($P < 0.001$) higher in the active attack group than in controls. Intracellular Ca^{2+} release in the cells was significantly ($P < 0.001$) lower in the remission (colchicine) group than in the attack group. Hence, we found that colchicine induced protective effects against fMLP-induced Ca^{2+} release. Intracellular Ca^{2+} release in neutrophil cells was significantly ($P < 0.001$) higher in the unremission group than in the control, active and remission groups. Hence, we found that colchicine did not induce protective effects against fMLP-induced Ca^{2+} release in the unremission group.

Effects of Colchicine on LP Levels in Serum and Neutrophils of Patients with FMF

Effects of colchicine on LP levels in PMN cells and serum are shown in Tables 1 and 2, respectively. LP levels in PMN cells and serum were significantly ($P < 0.05$) higher in the attack group than in controls. LP levels were significantly ($P < 0.05$) lower in the remission group than in the attack group. Hence, we found that colchicine induced protective effects against oxidative stress–induced LP levels in patients with a remission period. However, LP levels in PMN cells ($P < 0.01$) and serum ($P < 0.05$) were significantly higher in the unremission group than in the remission group.

Effects of Colchicine on GSH-Px, GSH, Vitamin A, Vitamine E and β-Carotene Values

Effects of colchicine treatment on GSH-Px, GSH, vitamin A, vitamin E and β-carotene values in PMN cells and serum are shown in Tables 1 and 2, respectively. Serum vitamin E and β-carotene concentrations were significantly ($P < 0.05$) lower in the attack group than in controls. Serum vitamin E ($P < 0.01$) and β-carotene ($P < 0.05$ and $P < 0.01$) concentrations were significantly higher in the remission group than in the attack, unremission and control groups. Hence, we found that colchicine induced antioxidant effects against oxidative stress–induced LP levels in patients with a remission period. However, GSH, GSH-Px and vitamin A values in PMN cells and serum in four groups did not change significantly.

Blood Biochemical Values

Effects of colchicine treatment on biochemical values are shown in Table 3. CRP levels ($P < 0.001$), ESR ($P < 0.001$) and leucocyte ($P < 0.05$) and neutrophil ($P < 0.001$) counts were significantly higher in the attack group than in controls, whereas CRP ($P < 0.01$), ESR ($P < 0.001$) and leucocyte ($P < 0.05$) values were significantly lower in the remission and unremission groups than in the attack group. Hemoglobin levels did not change within the four groups.

Discussion

The most important finding of this study is that serum and PMN LP levels as MDA and PMN $[Ca^{2+}]_i$ values were elevated in active patients with FMF compared to healthy subjects, whereas serum vitamin E and β-carotene concentrations in the active group were decreased by the disease. LP and $[Ca^{2+}]_i$ values were reduced to control levels by colchicine treatment, whereas serum vitamin E and β-carotene concentrations were increased by the treatment. To our knowledge, this is the first comparative study of colchicine on intracellular Ca^{2+} release and the oxidative and antioxidant system in human PMN cell medium. There is evidence that inflammatory FMF disease might impair antioxidant defense and increase LP as colchicine treatment itself increases oxidative stress and induces irreversible inflammatory changes in remission FMF patients.

FMF is characterized by activation of the inflammatory response system with increased production of procytokines (Guz et al. 2009). LP level as MDA is a major oxidative degradation product of membrane unsaturated fatty acid and has been shown to be biologically active with ROS properties (Halliwell 2006). In the present study, exposure to FMF enhanced PMN cells and serum LP in the human system. In FMF patients, upregulation of PMN and monocyte phagocytic activity over ROS production through oxidative burst was observed in the attack period (Kirkali et al. 2008). The overproduction of ROS leads to an increase in levels of LP as MDA, interferes with

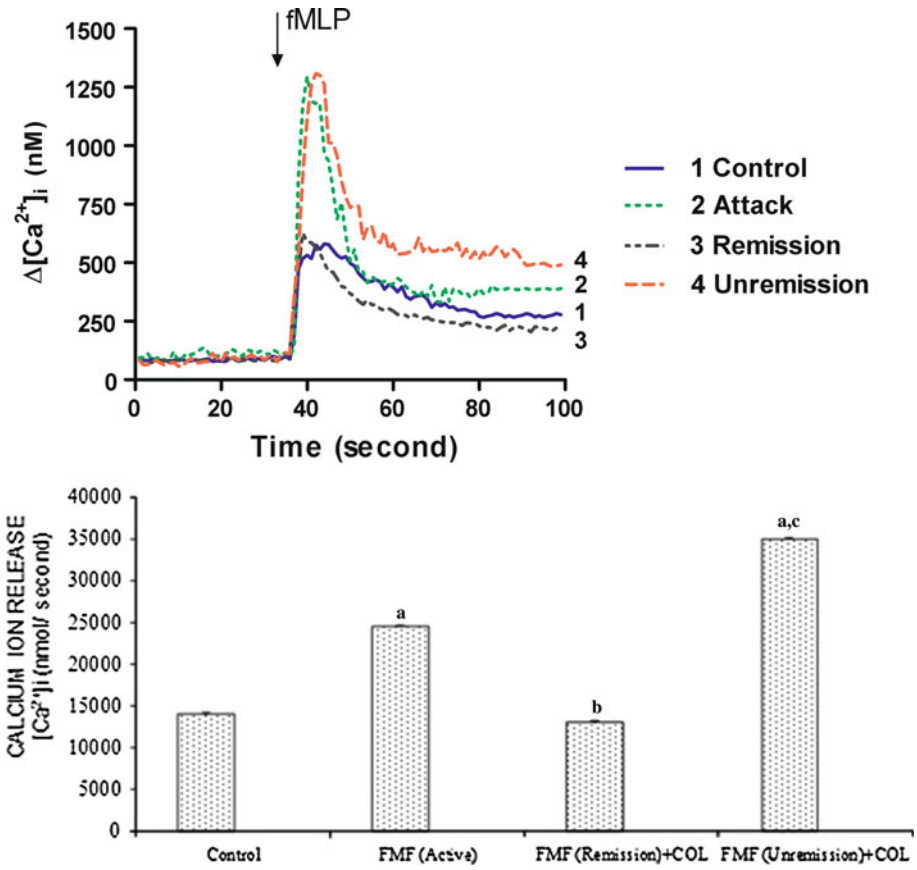

Fig. 1 Effects of colchicine (*COL*) treatment on intracellular Ca^{2+} release and $[Ca^{2+}]_i$ in PMNs of controls and FMF patients. Stimulation was performed by fMLP (1 μM). [a] $P < 0.001$ vs. control, [b] $P < 0.001$ vs. FMF (attack) group, [c] $P < 0.001$ vs. FMF (remission) group

Table 1 Effects of colchicine (COL) treatment on PMN LP, GSH and GSH-Px in controls and FMF patients ($n = 6$, mean ± SD)

Parameters	Control	FMF (attack) + COL	FMF (remission) + COL	FMF (unremission) + COL
LP (nmol/g protein)	22.88 ± 3.85	30.59 ± 5.95[a]	21.55 ± 4.61[c]	42.72 ± 3.66[b–d]
GSH (nmol/g protein)	12.15 ± 0.65	12.54 ± 0.99	14.22 ± 1.39	15.62 ± 0.90
GSH-Px (IU/g protein)	8.76 ± 1.50	8.30 ± 1.04	9.75 ± 1.33	10.82 ± 0.74

[a] $P < 0.05$ and [b] $P < 0.01$ vs. control, [c] $P < 0.05$ vs. FMF (attack), [d] $P < 0.01$ vs. FMF (remission)

Table 2 Effects of colchicine (COL) treatment on serum GSH, LP, vitamin A, β-carotene and vitamin E levels in controls and FMF patients (mean ± SD, $n = 6$)

Parameters	Control	FMF (attack)	FMF (remission) + COL	FMF (unremission) + COL
LP (nmol/ml)	1.71 ± 0.15	1.93 ± 0.12[a]	1.45 ± 0.10[a]	1.82 ± 0.12[d]
GSH (μmol/l)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
GSH-Px (IU/ml)	1.16 ± 1.15	1.22 ± 0.17	1.19 ± 0.16	1.24 ± 0.12
Vitamin A (nmol/l)	1.16 ± 1.15	1.22 ± 0.17	1.19 ± 0.16	1.24 ± 0.12
β-carotene (μmol/l)	2.47 ± 0.26	1.99 ± 0.37[a]	2.98 ± 0.42 [a,c]	2.03 ± 0.10[a,d]
Vitamin E (μmol/l)	11.89 ± 1.09	9.57 ± 1.80[a]	17.87 ± 1.57[b,c]	11.35 ± 1.29[e]

[a] $P < 0.05$ and [b] $P < 0.01$ vs. control, [c] $P < 0.01$ vs. FMF (attack), [d] $P < 0.05$ and [e] $P < 0.01$ vs. FMF (remission)

the structure and ratio of polyunsaturated fatty acids (Nazıroğlu 2007a, b) and causes loss of fluidity of the biological membrane. During the fever attack of FMF, there are usually overproduction of neutrophils, production of a brisk acute-phase protein such as serum amyloid A and substantial influx of PMNs to affected cells (Guz et al. 2009). As a result of these alterations, the biological membranes induce cytokine production (Freitas et al. 2009).

Table 3 Effects of colchicine (COL) treatment on biochemical values in controls and FMF patients (mean ± SD, $n = 6$)

Parameters	Control	FMF (attack) + COL	FMF (remission) + COL	FMF (unremission) + COL
CRP (hour)	7.10 ± 3.9	39.10 ± 3.50[c]	17.30 ± 1.79[b,e]	17.00 ± 4.20[f,g]
ESR (hour)	2.60 ± 0.80	106.31 ± 5.19[c]	7.10 ± 2.88[f]	5.10 ± 2.00[f]
Leucocytes ($\times 10^3/mm^3$)	7.93 ± 1.81	11.40 ± 3.12[a]	7.96 ± 2.29[d]	8.90 ± 2.14[d]
Neutrophil (%)	40.66 ± 1.18	84.50 ± 2.39[f]	50.50 ± 2.16	52.00 ± 1.53
Hemoglobin (g/dl)	13.80 ± 1.40	13.1 ± 1.40	14.60 ± 1.30	14.50 ± 1.50

[a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. control, [d] $P < 0.05$, [e] $P < 0.01$ and [f] $P < 0.001$ vs. FMF (attack), [g] $P < 0.05$ vs. FMF (remission)

On the other hand, elevation of LP induces phospholipase A_2, which changes receptor functions in the cell membranes, induces immune cells, leads to secretion of interleukins from T cells (Bréchard and Tschirhart 2008) and may increase LP. Treatment with colchicine effectively protected PMNs against FMF-induced neutrophil damage, as shown by increased serum vitamin E and β-carotene levels and decreased LP levels in serum and PMNs of FMF patients. It has been reported that colchicine treatment may partially suppress immune cells including T cells (Das et al. 2000). Suppression of immune cells by colchicine treatment may decrease oxidative stress. It may be concluded that colchicine may decrease LP levels and increase antioxidant values via reducing immune cells.

ROS act as subcellular messengers in such complex processes as mitogenic signal transduction, gene expression and regulation of cell proliferation when they are generated excessively or when enzymatic and nonenzymatic defense systems are impaired (Nazıroğlu 2007a, b). The major intracellular antioxidant enzyme GSH-Px detoxifies H_2O_2 to water and removes organic hydroperoxides (Halliwell 2006). Phagocytic cells are very vulnerable to oxidative stress because of elevated consumption of oxygen and the consequent generation of large amounts of ROS (Freitas et al. 2009). While colchicine is known to have antitubulin action and to inhibit mitosis, its possible beneficial effects in liver cells have been ascribed to wide-ranging properties. These include anti-inflammation action and inhibition of LP (Das et al. 2000). Results of the current study show an increase in vitamin E and β-carotene levels in response to colchicine treatment of active FMF patients.

For colchicine, a radical scavenging/antioxidant effect was suggested because it offered a simple way to explain certain phenomena that had no apparent connection to its well-known antimitotic activity. It is known to be effective in situations of acute inflammation. The antioxidant effect of colchicine seems unreasonable for structural reasons because it does not contain a functional group with pronounced electron-donating properties (Modriansky et al. 2002). In the current study, intracellular antioxidants such as GSH and GSH-Px in serum and PMN did not change by colchicine treatment, although concentrations of membrane-stabilizing antioxidants such as vitamin E and β-carotene were increased by colchicine treatment. Results from our study are complementary to the general view that colchicine has poor direct antioxidant properties. Its protective effects are probably mediated by a "membrane-stabilization effect" as suggested by Rojkind (1992). Similarly, Gurbuz et al. (2005) reported that GSH-Px activities did not change in proteinuric patients compared to nonproteinuric patients, although plasma LP levels in proteinuric FMF patients were increased.

Intracellular Ca^{2+} has been presented as a key regulator of cell survival, but this ion can also induce apoptosis in response to a number of pathological conditions (Nazıroğlu 2009). In addition, the mitochondria act as Ca^{2+} buffers by sequestering excess Ca^{2+} from the cytosol (Hajnóczky et al. 2006). Ca^{2+}-mobilizing agonists can effectively produce a rapid, simultaneous and reversible cessation of the movements of both the endoplasmic reticulum (ER) and mitochondria, which is strictly dependent on a rise in $[Ca^{2+}]_i$. This inhibition in mitochondrial motility reflects an increased mitochondrial Ca^{2+} uptake and, thus, enhances the local Ca^{2+}-buffering capacities of mitochondria, with important consequences for signal transduction (Rathore et al. 2008). Ca^{2+} overloading in mitochondria can induce an apoptotic program by stimulating the release of apoptosis-promoting factors like cytochrome *c* and by generating ROS due to respiratory chain damage (Hajnóczky et al. 2006; Nazıroğlu 2007a, b). Furthermore, mitochondria have been found to play a pivotal role in Ca^{2+} signaling (Bréchard and Tschirhart 2008). In fact, the release of Ca^{2+} from ER stores by IP_3 receptors has been implicated in multiple models of apoptosis as being directly responsible for mitochondrial Ca^{2+} overload (Ayub and Hallett 2004). Stored Ca^{2+} is crucial for a number of cellular functions, including signal-transduction cascades that respond to stress conditions (Bréchard and Tschirhart 2008). Colchicine has a direct regulatory effect on L-type voltage-gated Ca^{2+} channels in heart ventricle cells (Galli and DeFelice 1994; Rueckschloss and Isenberg 2004). Our results indicate that both blockade of Ca^{2+} uptake into

mitochondria and colchicine increasing $[Ca^{2+}]_i$ in the remission group were able to decrease LP mediated by oxidative stress, which released Ca^{2+} from intracellular stores.

The antioxidant enzyme system inherent in the cellular defense system is the most important defense mechanism against ROS. GSH and GSH-Px together act as antioxidants and have a preventive effect against extensive production of hydrogen peroxide by PMN cells (Bréchard and Tschirhart 2008). Limited colchicine and FMF results on GSH and GSH-Px values in patients and experimental animals are conflicting. Glutathione reductase is an antioxidant enzyme which reduces glutathione disulfide to the sulfhydryl form GSH, and glutathione reductase activity was increased dose-dependently by colchicine (Tandogan and Ulusu 2010). Gurbuz et al. (2005) reported that GSH-Px activities did not show statistically significant differences between FMF patients with and those without proteinuria. Modriansky et al. (2002) reported that colchicine in rat liver did not affect GSH and GSH-Px values. In the current study, GSH and GSH-Px activities did not significantly change in the four groups. Adaptive antioxidant responses of GSH-Px were accompanied by GSH upregulation (Eroglu et al. 2010).

In conclusion, we firstly indicated antioxidant reduction and Ca^{2+} release in neutrophils and serum of patients with FMF. Our results suggest that the persistent activation of neutrophils may be the cause of oxidative damage. Colchicine treatment induced a protective effect on oxidative stress, the antioxidant redox system and Ca^{2+} release in serum and PMNs of remission FMF patients. Our current study on PMN cells taken together with in vitro studies (Galli and DeFelice 1994; Modriansky et al. 2002) on colchicine suggest, therefore, an unappreciated therapeutic potential for this drug in inflammatory diseases such as FMF that are characterized by oxidative stress.

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Conflicts of interest The authors declare that there are no conflicts of interest.

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