MÜSBED 2013;3(4):200-207
DOI: 10.5455/musbed.20131215110852

Araştırma / Original Paper

The Effect of Some Antibiotics on Polymorphonuclear Leukocyte (PMN) Functions and PMN's Myeloperoxidase Activity, Glutathione and Malondialdehyde Levels of Patients with Type 2 Diabetes Mellitus In Vitro

Pervin Rayaman[1], Erkan Rayaman[1], Adile Çevikbaş[1], Refik Demirtunç[2], A. Özer Şehirli[3], Ümran Soyoğul Gürer[1]

[1]Marmara Üniversitesi Eczacılık Fakültesi, Farmasötik Mikrobiyoloji AD, İstanbul - Türkiye
[2]Haydarpaşa Numune Eğitim ve Araştırma Hastanesi, İç Hastalıkları Kliniği, İstanbul - Türkiye
[3]Marmara Üniversitesi Eczacılık Fakültesi, Farmakoloji AD, İstanbul - Türkiye

Yazışma Adresi / Address reprint requests to: Pervin Rayaman,
Marmara Üniversitesi Eczacılık Fakültesi, Farmasötik Mikrobiyoloji AD, Tıbbiye Cd. No: 49 34668, İstanbul - Türkiye
Elektronik posta adresi / E-mail address: pgocer2000@yahoo.com
Kabul tarihi / Date of acceptance: 15 Aralık 2013 / December 15, 2013

ÖZET

Bazı antibiyotiklerin Tip 2 diabetes mellitus'lu hastaların polimorfonüklear lökosit (PNL) fonksiyonları ve PNL'lerin miyeloperoksidaz aktivitesi, glutatyon ile malondialdehit düzeyleri üzerine in vitro etkisi

Amaç: Çalışmamızda diabetes mellitus (DM)'lu hastaların polimorf nüveli lökosit (PNL) fonksiyonları ile sağlıklı gönüllülerin miyeloperoksidaz (MPO) aktivitesi, glutatyon (GSH) ve malondialdehit (MDA) düzeylerini karşılaştırıp arasındaki ilişkiyi saptamayı amaçladık. Bunun yanında siprofloksasin, rifampisin ve doksisiklinin PNL fonksiyonları, MPO aktivitesi ve GSH ile MDA düzeyleri üzerine immunomodülatör etkilerini araştırdık.
Yöntemler: Çalışmamızda Tip 2 diyabeti olan 13 hasta ve 13 sağlıklı gönüllü yer almaktadır. Antibiyotiklerin PNL fonksiyonları üzerine olan etkileri in vitro koşullarda araştırıldı. PNL'ler ($1x10^7$ hücre/ml) venöz kandan Ficoll-hypaque gradient santrifügasyon metodu kullanılarak izole edildi. PNL'lerin MPO aktivitesi modifiye O-dianisidin yöntemi, GSH miktarı Ellman yöntemi ve MDA düzeyleri ise Beuge yöntemi kullanılarak belirlendi.
Bulgular: DM'lu hasta PNL'lerinin fagositik ($p<0.05$) ve hücre içi öldürme aktivitesi ($p<0.01$) sağlıklı gönüllülere göre anlamlı olarak azaldı. Siprofloksasin sağlıklı gönüllü ve tüm hasta PNL'lerinin fagositik ve hücre içi öldürme aktivitesini kontrol değerlere göre anlamlı olarak artırdı ($p<0.01$ ve $p<0.01$). Rifampisin, DM'lu hasta PNL'lerinin hücre içi öldürme aktivitesini anlamlı olarak artırdı ($p<0.05$). Rifampisin ve doksisiklin ise, sağlıklı gönüllü PNL'lerinin fagositik ve hücre içi öldürme aktivitesini kontrol değerlerine göre anlamlı olarak azalttı ($p<0.05$). Siprofloksasin (2.5 µg/ml) sağlıklı gönüllülerin ($p<0.01$) ve hastaların MPO aktivitesini ve MDA düzeyini ilaçsız kontrole göre anlamlı olarak artırdı ($p<0.01$). Ancak aynı ilaç DM'lu hasta PNL'lerinin GSH düzeyini anlamlı olarak azalttı ($p<0.05$).
Sonuçlar: İmmün sistemi bozulmuş Tip 2 DM'lu hastalardaki ve sağlıklı gönüllerdeki MPO aktivitesi ile PNL fonksiyonları arasındaki ilişkinin önemi siprofloksasin ile hücresel immün sistemin desteklenmesi ve PNL'lerin MPO aktivitesi ile beraber PNL aktivitelerinin artışı ile gösterildi.
Anahtar sözcükler: Diabetes mellitus, miyeloperoksidaz, fagositoz, hücre içi öldürme aktivitesi, antibiyotikler

ABSTRACT

The effect of some antibiotics on polymorphonuclear leukocyte (PMN) functions and PMN's myeloperoxidase activity, glutathione and malondialdehyde levels of patients with type 2 diabetes mellitus in vitro

Objective: In this study, our objective was to compare PMN functions and myeloperoxidase (MPO) activity, glutathione (GSH) and malondialdehyde (MDA) levels and assess the relationship between PMN functions and enzyme levels in patients with type 2 diabetes mellitus (T2DM) and healthy volunteers. We also examined the immunomodulatory effects of ciprofloxacin, rifampicine and doxycycline on PMN functions, MPO activity and GSH and MDA levels.
Methods: Thirteen patients with T2DM and 13 healthy volunteers were included in the study. The effect of antibiotics on PMN functions were investigated in vitro. PMN's ($1x10^7$cells/ml) were isolated from venous blood by Ficoll-hypaque gradient centrifugation method. MPO activity was assayed by modified O-dianisidine method. GSH level was determined using Ellman's method and MDA level by Beuge's method.
Results: The PMN's phagocytic ($p<0.05$) and intracellular killing activity ($p<0.01$) of T2DM patients significantly decreased when compared with healthy volunteers. Ciprofloxacin increased PMN's phagocytic ($p<0.01$) and intracellular killing activity ($p<0.01$) of T2DM patients and healthy volunteers when compared with drug-free controls. Rifampicine increased PMN's intracellular killing activity of patients with T2DM ($p<0.05$). Rifampicine and doxycycline decreased PMN's phagocytic and inracellular activity of healthy volunteers ($p<0.05$). Ciprofloxacin increased PMN's MPO activity ($p<0.01$) and MDA levels of T2DM patients ($p<0.01$) and healthy volunteers ($p<0.01$). However, it decreased PMN's GSH levels of T2DM patients ($p<0.05$).
Conclusions: The importance of the relationship between MPO activity and PMN functions was shown by the support of cellular immunity and increase of PMN's MPO activity and PMN functions with ciprofloxacin in healthy volunteers and T2DM patients whose immune system is deteriorated.
Key words: Diabetes mellitus, myeloperoxidase, phagocytosis, intracellular killing activity, antibiotics

INTRODUCTION

Polymorphonuclear leukocytes (PMN) have an important role in the cellular host defence mechanism against various microorganisms. It is known that PMN functions are deteriorated in type 2 diabetes mellitus (T2DM) patients; thus, they become more susceptible against infections. The deterioration of PMN functions, expecially the decrease in their intracellular killing activity of these patients was demonstrated by several studies (1,2).

In our previous study, the PMN's intracellular killing activity of T2DM patients was shown to be decreased significantly. However, the cause of this abnormability seen in these patients is still unknown (1).

When neutrophils meet bacteria or other particules, the oxygen consumption increases along with superoxide (O_2.-) and hydrogen peroxide (H_2O_2) formation. Hydrogen peroxide, myeloperoxidase (MPO)- released from azurophilic granules of neutrophils,- and cloride (Cl^-) result in the formation of the oxidant and antimicrobial agent hypoclorous acid (HOCl) to kill intracellular microorganisms (2). Several studies have shown decreased MPO activity in T2DM patients (4,5). This suggests that changes in the activity of this enzyme deteriorates PMN functions and may affect the susceptibility to infections and immunocompetence of T2DM patients. It was also shown that there was a correlation between the HbA1c concentrations and MPO activity in these patients (4).

Glutathione (GSH) is a major antioxidant in the non-enzymatic antioxidant defence system. By converting oxidants such as hydrogen peroxide and superoxides to other oxidative forms, GSH becomes a potent intracellular and extracellular antioxidant. It controls the epithelial cell functions by invigorating the antioxidant defence (6,7). GSH levels indicate the level of antioxidant defence level of the body and blood GSH levels were found to decrease in T2DM patients when compared to controls (8).

As an indicator of lipid peroxidation, malondialdehyde (MDA) is used in the measurement of oxidative stress. It was shown that serum MDA levels of T2DM patients particularly in those with complications significantly increased when compared to controls (8).

As the cause of oxidative stress in T2DM patients is not exactly determined, in this study, we aimed to find out the relationship between PMN functions (phagocytosis and intracellular killing activity) and MPO activity, GSH and MDA levels of these cells in T2DM patients. By reducing the enzyme and antioxidants levels, the drugs and antibiotics used in the chronic treatment of these patients could make them more susceptible to infectious diseases. The antimicrobial drugs used in the treatment of infections in DM patients could negatively effect or increase the enzyme levels or activities. It is important to choose the proper antibiotic in order to stimulate the deteriorated PMN functions in T2DM patients. Thus, we also aimed to find out the effect of ciprofloxacin, rifampicine and doxycycline on PMN functions and MPO activity, GSH and MDA levels of T2DM patients and healthy volunteers in vitro.

METHODS

Patients

Blood was collected from 13 patients who attended Haydarpaşa Numune Training and Research Hospital. Thirteen patients (5 males and 8 females) with type 2 DM, whose age range was 29-48 years (mean age 40.31) and HbA1c>8% were included in the study. The average duration after diagnosis of DM was 3.27 years and the average fasting blood glucose level was 179 mg/dl. Exclusion criteria were existence of an infectious, rheumatic, malignant or any other systemic disease.

Volunteers

Control group consisted of 13 healthy volunteers whose age range was 29-53 years (mean age 39.69) None of them had any disease, pregnancy and used any drugs. This study protocol was approved by the Marmara University Ethics Committee.

Isolation of PMNs

Peripheral blood samples (10 ml) from patients and healthy volunteers were drawn with ethylenediaminetetraacetic acid (EDTA). PMNs from venous blood with EDTA ($1x10^7$ cells/ml) were isolated by Ficoll-hypaque gradient centrifugation method as described previously (9,10).

Measurement of PMN activities

Phagocytosis and intracellular killing activity were assayed by modifying Alexander's method (11). In the modified method, Ficoll was used instead of dextran and PMNs were counted by microscope instead of standard pour plate technique. PMN viability was assayed as 98% by trypane blue staining and PMNs were suspended in Hank's Buffered Salt Solution (HBSS) and cell density was adjusted by dilution ($1x10^7$ cell/ml) (9,12).

A clinical strain of *Candida albicans* was used to determine the phagocytic and intracellular killing activity of PMNs. *C. albicans's* viability was assayed as greater than 98% by methylene blue staining. PMNs were suspended in HBSS and incubated at 37°C for 30 minutes in a shaking incubator. In a separate tube, *C. albicans* were suspended in HBSS and then an aliquoat of sterile human serum (1:4) was added to induce opsonization and the mixture was incubated at 37°C for 30 minutes. Subsequently, opsonized yeast cells were added to the PMNs and the final mixture contained $5x10^6$ PMNs/ml and 5x106 yeasts/ml. Dead yeast cells were determined by adding 0.01% methylene blue (1:1 ratio) in the last 5 minutes of the incubation. The phagocytic activity was determined by the percentage of PMN's that had phagocytosed yeast cells. Intracellular killing activity was determined by the percentage of PMNs that included killed yeast cells (9,10,13). PMN suspensions were stored at -20˚C to measure PMN's MPO activity, GSH and MDA levels of patients and healthy volunteers.

Antibiotics

In vitro effect of ciprofloxacin (2.5 µg/ml), rifampicine (7µg/ml) and doxycycline (2.5 µg/ml) at therapeutic serum concentrations on PMN functions was investigated. Ciprofloxacin and doxycycline were prepared as stock solutions at therapeutic serum concentrations in distilled water. Rifampicine was prepared as stock solution at therapeutic serum concentrations in methanol.

Preparation of PMN homogenates

The PMN suspension was frozen at -80°C and thawed six times, then homogenized using a motor driven Teflon-glass homogenizer (9000 rpm for 5 min at 0°C) (14).

Measurement of the MPO activity in PMNs

MPO activity was determined by a modification of the o-dianisidine method. The protein content of the homogenate was measured by Spectronic-UV 120 spectrophotometer using Lowry's method (15). The assay mixture, in a cuvette of 1 cm path length, contained 0.3 mL 0.1M phosphate buffer (pH 6.0), 0.3mL 0.01M H2O2, 0.5mL 0.02M O-dianisidine (freshly prepared) in deionized water and 10µL PMN homogenate in a final volume of 3 mL. The PMN homogenate was added and the change in absorbance at 460 nm was followed for 10 min. All measurements were carried out in duplicate. One unit of MPO was defined as that giving an increase in absorbance of 0.001 per min and specific activity was given as IU/mg protein (14).

Measurement of the MDA and GSH levels of PMNs

PMN samples were homogenized with ice-cold 150 mm KCl for the determination of MDA and GSH levels. The MDA levels were assayed for the products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation, as described previously (16). Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and the data were expressed as nmol/mg. GSH measurements were performed using a modification of the Ellman procedure (17). Briefly, after centrifugation at 2000 × g for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/L $Na_2HPO_4.2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$. The data were expressed as µmol /mg (16,17).

Statistical analysis

The results were expressed using means ± SD. Statistical analyses were performed using Mann Whitney U and Wilcoxon Signed Ranks tests. P values less than or equal to 0.05 were considered to be statistically significant.

Table 1: Phagocytic activity (PA), intracellular killing activity (IKA), myeloperoxidase (MPO) activity, glutathione (GSH) and malondialdehyde (MDA) levels of polymorphonuclear (PMN) cells in patients with type 2 diabetes mellitus and healthy volunteers

Group	PA (%)	IKA (%)	MPO (U/mg protein)	GSH(µmol/mg protein)	MDA (nmol/mg protein)
Diabetes mellitus n=13	39.54±1.99*	1.54±0.18**	227.44± 16	2.01 ± 0.06	2.03±0.89
Healthy volunteers n=13	48.31±5.27	6.54±1.39	275.96 ± 33	1.94 ± 0.07	2.22 ± 0.50

*p<0.05, **p<0.001
Statistical analysis was done using Mann Whitney U test and the data were shown in mean ± SD.

Table 2: Comparison of the effect of antibiotics on phagocytic activity (PA), intracellular killing activity (IKA) in drug-free patients with type 2 diabetes mellitus and healthy volunteers

Group	n	Ciprofloxacin (2.5 µg/ml)		Rifampicine (7µg/ml)		Doxycycline (2.5 µg/ml)	
		PA (%)	IKA (%)	PA (%)	IKA (%)	PA (%)	IKA (%)
Drug-free	13	39.54±1.99	1.54±0.18	39.54±1.99	1.54±0.18	39.54±1.99	1.54±0.18
Diabetes mellitus		53.92±1.90*	4.15±0.48*	41.23±1.88	1.62±0.42**	41.23±1.88	1.62±0.42
Drug-free	13	48.31±5.27	6.54±1.39	48.31±5.27	6.54±1.39	48.31±5.27	6.54±1.39
Healthy volunteers		63.54±1.89*	9.92±0.35*	39.08±2.39**	5.62±0.35**	38.15± 1.82**	5.69±1.62**

*p<0.01, **p<0.05
Statistical analysis was done using Wilcoxon Signed Ranks test and the data were shown in mean± SD.

Table 3: Comparison of the effect of antibiotics on phagocytic activity (PA), intracellular killing activity (IKA), myeloperoxidase (MPO) activity, glutathione (GSH) and malondialdehyde (MDA) levels of polymorphonuclear (PMN) cells in drug-free patients with type 2 diabetes mellitus and healthy volunteers

	MPO (U/mg protein)		GSH (µmol/mg protein)		MDA (nmol/mg protein)	
Group	Diabetes mellitus (n=13)	Healthy (n=13)	Diabetes mellitus (n=13)	Healthy (n=13)	Diabetes mellitus (n=13)	Healthy (n=13)
Drug-free (2.5 µg/ml)	227.44±16	275.96±33	2.01±0.06	1.94±0.07	2.03±0.89	2.22±0.50
ciprofloxacin	343.60±17*	391.58±30*	1.32±0.11*	2.19±0.13*	2.66±0.52*	2.68±0.40*
Drug-free	227.44±16	275.96±33	2.01±0.06	1.94±0.07	2.03±0.89	2.22±0.50
rifampicine (7µg/ml)	238.66±17	272.94±30	1.82±0.07**	1.87±0.11	1.99±0.50	2.28±0.24*
Drug-free	227.44±16	275.96±33	2.01±0.06	1.94±0.07	2.03±0.89	2.22±0.50
doxycycline (2.5 µg/ml)	229.14±16	284.33±27	1.84±0.05**	1.81 0.12	1.81±1.40	2.18±0.39

*p<0.01, **p<0.05
Statistical analysis was done using Wilcoxon Signed Ranks test and the data were shown in means ± SD.

RESULTS

The phagocytic ($p<0.05$) and intracellular killing activity ($p<0.01$) of PMNs in T2DM patients significantly decreased when compared with healthy volunteers (Table 1). There is no statistical difference between T2DM and healthy volunteers in terms of MPO activity, GSH and MDA levels of PMNs (Table 1). Ciprofloxacin (2.5 µg/ml) increased PMN's phagocytic ($p<0.01$) and intracellular killing activity ($p<0.01$) in T2DM patients and healthy volunteers when compared with drug-free controls (Table 2). While rifampicine did not significantly change the PMN's phagocytic activity in patients with T2DM when compared with drug-free controls it significantly increased PMN's intracellular killing activity ($p<0.05$). However, doxycycline did not significantly change PMN functions in the same group when compared with drug-free controls, but the same antibiotics decreased PMN's phagocytic and intracellular killing activity in healthy volunteers when compared with drug-free controls ($p<0.05$) (Table 2). Ciprofloxacin (2.5 µg/ml) increased MPO activity ($p<0.01$) and MDA levels of PMNs in T2DM patients ($p<0.01$) and healthy volunteers ($p<0.01$) when compared with drug-free controls. However, while it decreased the GSH levels of PMNs in T2DM patients ($p<0.01$) and it increased the GSH levels of the cells in healthy volunteers ($p<0.01$) when

compared with drug-free controls (Table 3). Rifampicine (7 µg/ml) did not affect the PMN's MPO activity when compared with drug-free controls. It decreased GSH levels in patients with T2DM when compared with drug-free controls ($p<0.05$). However, it increased the MDA levels of the PMNs in healthy volunteers when compared with drug-free controls ($p<0.05$) (Table 3). Doxycycline (2.5 µg/ml) did not significantly affect the MPO activity and MDA levels of the cells in patients with T2DM and healthy volunteers when compared with drug-free controls ($p>0.05$). However, it decreased the GSH levels of patients with T2DM when compared with drug-free controls ($p<0.05$) (Table 3).

DISCUSSION

It is known that some of the antibiotics used in the treatment of infections could overcome bacterial resistance and some well-known antibiotics are able to show immunomodulatory effects by interaction with neutrophils (18). Additionally, while some antibiotics suppress PMN functions, the others do not affect them.

Patients with DM have a higher infective risk than healthy volunteers, with more frequent and severe infections. In particular, there is PMN dysfunction including chemotaxis, phagocytosis, bacterial killing and intracellular killing (19). As, the phagocytic and intracellular killing activity of PMNs in T2DM patients significantly decreased when compared with healthy volunteers in our study, it must be considered that it is important to take note of the antibiotics and other drugs which are used in the treatment of diabetes. Ciprofloxacin, used as an immunomodulatory antibiotic model in our study, significantly increased both PMN functions in patients with T2DM when compared with drug-free controls (Table 2). Although the decrease of MPO activity in patients T2DM in our study was statistically insignificant, it might have negatively affected the phagocytic and intracellular killing activity of the PMNs. MPO activity of the PMNs in patients with T2DM ($p<0.001$) and related with it, their phagocytic ($p<0.001$) and intracellular killing activity ($p<0.001$) significantly increased after immunomodulatory ciprofloxacin application. Since ciprofloxacin significantly increased both PMN functions and MPO activity in patients with T2DM, in our opinion there is a relationship between PMN functions and MPO activity. If ciprofloxacin or other antibiotics which possess the same useful effect and have immunomodulatory effects during the therapy of the infections in DM patients, using this antibiotic in their therapy protocole would strengthen the PMN functions leading to more beneficial results.

The disfunctions seen in PMN functions of patients with DM are related to glycemia and glycated hemoglobin (19). Marhoffer et al. (20) have shown that phagocytic and intracellular killing activity of the PMNs in DM patients significantly decreased when compared with healthy controls ($p<0.001$). The results of Marhoffer et al. (20) confirm the results of our study. The investigators determined significantly negative correlations for fasting blood-glucose concentrations ($p=0.001$) as well as glycosylated hemoglobin (HbA1c) ($p=0.03$) and a highly positive correlation for intracellular killing ($p=0.0001$) by linear regression analysis. They declared that impaired PMN functions in patients with DM were inversely related to the degree of metabolic control of diabetes. These findings suggest inhibitory effects of hyperglycemia on PMN functions, thus contributing partly to altered host defense in DM.

Sato et al. (4) have stated that the increase of glucose transport in PMNs of patients with DM could negatively effect their phagocytic activity. The same investigators have shown that MPO activity of patients with DM significantly decreased when compared with healthy controls. Sato et al. demonstrated that there was a correlation between MPO activity and HbA1c levels in patients with DM. The investigators have shown that when negative correlation between MPO activity and HbA1c levels of patients with DM lasts for a long time, the MPO activity of the PMNs decreases.

In their study, Uchimura et al. (5) claimed that the correlation between HbA1c and MPO activity was not statistically significant. The investigators have found that MPO activity in neutrophils of patients with DM was lower than controls ($p< 0.05$). Wykretowicz et al. (2) have shown that intracellular killing activity and MPO activity of the PMNs in patients with DM sinificantly decreased ($p<0.01$, $p<0.01$). The researchers have claimed that the hyperglycemia seems to be one of the most likely cause of the observed disturbances. They claimed that when MPO decreases there is increased glucose metabolism and rise in NADPH. Consequently, increase in O_2.- and hydrogen peroxide formation and degranulation in patients with DM

lead to decrease in MPO activity.

We were not able to find a study showing a result, in which a drug would stimulate the MPO activity in patients with T2DM which is decreased in these patients and also by the increase in their MPO activity there is increase in their PMN functions.

In our study, the PMN functions together with MPO activity in patients with T2DM were found to decrease when compared with healthy volunteers. The studies on this subject confirm our results. Beside these results we have shown that an immunomodulatory antibiotic increasing PMN's MPO activity increases PMN functions and by increasing MPO activity in patients whose MPO activity is low, PMN functions would also increase. The increase in PMN functions in T2DM patients by the use of an immunomodulatory antibiotic such as ciprofloxacin is an important finding of our study. Ciprofloxacin, used as an immunomodulatory antibiotic model in our study, significantly decreased the GSH level of the PMNs in patients with T2DM ($p<0.01$). However, it significantly increased the MDA level of the cells ($p<0.01$).

The increase of MDA which is an indicator of lipid peroxidation in healthy volunteers and patients, might be the result of the over increase of MPO activity by ciprofloxacin. Ciprofloxacin significantly increased the MDA level of the PMNs in healthy volunteers and patients with T2DM. Probably, this is because ciprofloxacin signicantly over-stimulated the MPO activity in PMNs under in vitro conditions. It is possible that excessively increased MPO activity escalated the lipid peroxidation in the cell. This escalation might lead to an increase in the MDA levels of the cells. This needs further clinical and in vitro investigations.

Gürer et al. (13) have shown that ciprofloxacin significantly increased the phagocytic activity of the PMNs in elderly patients ($p=0.002$). Zinc (Zn) supplementation (15 mg/day) for 1 month together with ciprofloxacin have significantly increased the intracellular killing activity together with phagocytic activity of PMNs in elderly patients. The same investigators have found that ciprofloxacin, at the same dose significantly increased the phagocytic ($p=0.05$) and intracellular killing activity ($p<0.05$) of PMNs in these patients before Zn supplementation, but they have not investigated the beneficial effect on the MPO activity, GSH and MDA levels in these cells.

Altınordulu and Erarslan (21) have found that serum MDA levels of chicks, which were given ciprofloxacin, enrofloxacin and norfloxacin (10 mg/kg/day/orally) for 1, 3, 5 and 7days, insignificantly increased when it was compared with that of control MDA.

We found that ciprofloxacin significantly increased MDA levels of PMNs in T2DM patients with and healthy volunteers. In our opinion, these data have to be supported by clinical studies. The in vivo study on chicks give us an impression that ciprofloxacin could increase the MDA levels in clinical studies. Considering the teratogenic effects of ciprofloxacin on fetus during the first 3 months of pregnancy, these adverse effects may result from the escalation of lipid peroxidation which increased MDA levels of PMNs after ciprofloxacin.

While ciprofloxacin significantly decreased phagocytic activity of PMNs in healthy volunteers ($p<0.05$), it did not affect their intracellular killing activity. The same antibiotic did not significantly effect both PMN functions, MPO activity, GSH and MDA activity in T2DM patients.

In their study Daşdelen et al. (22) have shown that rifampicine (7 µg/ml) significantly decreased the phagocytic ($p<0.05$) and intracellular killing activity ($p<0.01$) of PMNs in healthy volunteers when compared with drug-free values. Gürer et al. (13) have shown that rifampicine significantly increased the phagocytic activity of PMNs in elderly patients ($p<0.05$) when compared with drug-free values before zinc supplementation. These results support our data.

In our study, rifampicine significantly decreased the phagocytic activity and intracellular killing activity of PMNs in healthy volunteers ($p<0.05$) when compared with drug-free values. These resuts are in accordance with the results of Daşdelen et al. (22). Rifampicine (7 µg/ml) did not signifficantly affect the MPO activity of PMNs in T2DM patients and healthy volunteers when compared with drug-free controls. However, it decreased GSH levels of the cells in T2DM patients when compared with drug-free controls ($p<0.05$). However, rifampicine increased the MDA levels of PMNs in healthy volunteers when compared with drug-free controls ($p<0.05$). Since rifampicine decreased both PMN functions in healthy volunteers in our study and in above-mentioned studies (13,22), it might have negatively affect the immune system. Additionally, the deterioration of the PMN functions by rifampicine is accompanied by the

increase of MDA levels which shows the presence of lipid peroxidation during oxidative stress. Also, the decrease of the GSH levels in T2DM patients with rifampicine shows that this antibiotic decreased the antioxidant defence of the body during diabetes.

Daşdelen et al. (22) have shown that doxycycline (2.5 µg/ml) significantly decreased the phagocytic and intracellular killing activity of PMNs ($p<0.05$) in healthy volunteers when compared with drug-free values. Similarly, in our study doxycycline (2.5 µg/ml) decreased the phagocytic activity and intracellular killing activity ($p<0.05$) of PMNs in healthy volunteers when compared with drug-free values. Conversely, the same antibiotic both PMN functions of patients with T2DM when compared with drug-free controls. Additionally, in our study doxycycline (2.5 µg/ml) did not affect the MPO activity and MDA levels of PMNs in T2DM patients. However, the same antibiotic just like rifampicine significantly decreased the GSH levels in T2DM patients when compared with drug-free controls ($p<0.05$).

In this study, the importance of the relationship between MPO activity and PMN functions was supported by the increase in MPO activity of PMNs and PMN functions with ciprofloxacin in healthy volunteers and T2DM patients whose immune system is deteriorated. However, the relationship between MDA levels and PMN functions was shown by the decreases in the cellular immunity and the increase in the MDA level of PMNs by rifampicine. That is why ciprofloxacin could be the preferential antibiotic during the treatment of these patients. However, the data on rifampicine and doxycycline need to be clinically supported by other studies. Beside the beneficial effects of ciprofloxacin, since there might be cellular damage when MDA increases in and PMNs while ciprofloxacin treatment invigorates immune system cells in these patients, and we believe that by the addition of various antioxidants to the treatment and by proving our in vitro results by clinical investigations which include more subjects we might bring more successful results in patients with diabetes mellitus.

Acknowledgements

The authors are thankful to the Marmara University, Scientific Research Commission (SAG-DKR-060907-0184) and all volunteers.

REFERENCES

1. Oberg G, Hällgren R, Moberg L, Venge P. Bactericidal proteins and neutral proteases in diabetes neutrophils. Diabetologia. 1986; 29(7): 426-429.
2. Wykretowicz A, Wierusz-Wysocka B, Wysocki J, Szczepanik A, Wysocki H. Impairment of the oxygen-dependent microbicidal mechanisms of polymorphonuclear neutrophils in patients with type 2 diabetes is not associated with increased susceptibility to infection. Diabetes Res Clin Pract. 1993; 19(3): 195-201.
3. Göçer P, Gürer US, Erten N, Palanduz S, Rayaman E, Akarsu B et al. Comparison of polymorphonuclear leukocyte functions in elderly patients and healthy young volunteers. Med Princ Pract. 2005; 14(6): 382-385.
4. Sato N, Shimizu H, Suwa K, Shimomura Y, Kobayashi I, Mori M. MPO activity and generation of active O2 species in leukocytes from poorly controlled diabetic patients. Diabetes Care. 1992; 15(8): 1050-1052.
5. Uchimura K, Nagasaka A, Hayashi R, Makino M, Nagata M, Kakizawa H et al. Changes in superoxide dismutase activities and concentrations and myeloperoxidase activities in leukocytes from patients with Diabetes mellitus. Diabetes Complicat. 1999; 13(5-6): 264-70.
6. Beeh KM, Beier J, Koppenhoefer N, Buhl R 2004. Increased glutathione disulfide and nitrosothiols in sputum supernatant of patients with stable COPD. Chest. 2004; 126(4): 1116-1122.
7. Shurtz-Swirski R, Sela S, Herskovits AT, Shasha SM, Shapiro G, Nasser L et al. Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. Diabetes Care. 2001; 24(1): 104-110.
8. Gupta A, Tripathi AK, Tripathi RL, Madhu SV, Banerjee BD. Advanced glycosylated end products-mediated activation of polymorphonuclear neutrophils in Diabetes mellitus and associated oxidative stress. Indian J Biochem Biophys. 2007; 44(5): 373-378.
9. Richardson MD, Scott G, Shankland GS. Effects of ciloftragin on phagocytosis and intracellular killing of candida albicans by human neutrophils. Eur J Clin Mic Infec Dis. 1996; 11(1): 22-26.
10. Işık H, Cevikbaş A, Gürer US, Kiran B, Uresin Y, Rayaman P et al. potential adjuvant effects of nigella sativa seeds to ımprove specific ımmunotherapy in allergic rhinitis patients. Med Princ Pract. 2010; 19(3): 206-211.
11. Alexander JW, Windhorst DB, Good RA. Improved tests for the evaluation of neutrophil function in human disease. J Lab Clin Med. 1968; 72(1): 36-48.
12. Rayaman P, Erçağ E, Gürer ÜS, Erten N, Rayaman E, Üzer A et al. The effect of zinc supplementation on polymorphonuclear leukocyte functions of elder hypertensive patients and healthy young volunteers. Turkish J Pharm Sci. 2009; 6: 31-43.

13. Gürer US, Göçer P, Erçağ E, Erten N, Rayaman E, Akarsu B et al . Effects of some antibiotics on polymorphonuclear leukocyte functions of elderly patients in vitro before and after zinc suplementation. Int. Immunopharmacol. 2006; 6: 808-816.

14. Kurutas EB, Cetinkaya A, Bulbuloglu E, Kantarceken B. Effects of antioxidant therapy on leukocyte myeloperoxidase and cu/zn-superoxide dismutase and plasma malondialdehyde levels in experimental colitis. Mediators Inflamm. 2005; 14(6): 390-394.

15. Lowry OH, Rosebrough NJ, Farr Al, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951; 193(1): 265-275.

16. Beutler E. Glutathione in Red Blood Cell Metabolism. A manual of biochemical methods. Grune&Stratton, New York, 1975.

17. Beuge JA, Aust SD. Microsomal lipid peroxidation. Meth Enzymol. 1978; 52: 302-311.

18. Boogaerts MA, Malbrain S, Scheers W, Verwilghen RL. Effects of quinolones on granulocyte function in vitro. Infection. 1986; 14(4): 258-262.

19. Hopps E, Camera A, Caimi G. Polimorphonuclear leukocytes and Diabetes mellitus. Minerva Med. 2008; 99(2): 197-202.

20. Marhoffer W, Stein M, Mäser E, Federlin K. Reduced phagocytic capacity of circulating granulocytes in Diabetes mellitus. Immun Infekt. 1992; 20(1): 10-12.

21. Altınordulu Ş. ve Eraslan G. Effects of some quinolone antibiotics on malondialdehyde levels and catalase activity in chicks. Food Chem Toxicol. 2009; 47(11): 2821-2823.

22. Daşdelen N, Soyoğul Gürer Ü, Çevikbaş A, İmamoğlu Ç, Johansson C. PNL fonksiyonlarını inhibe eden ve immünomodülatör etki gösteren antibiyotiklerin kombine kullanımlarının insan PNL fonksiyonları üzerine etkisinin in vitro araştırılması. Türk Mikrob Cem Derg. 1999; 29: 17-22.