Short report

Effect of the treatment of brucellosis on leukocyte superoxide dismutase activity and plasma nitric oxide level

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Abstract

Background: The mechanisms by which brucellae evade intracellular killing by polymorphonuclear leukocytes are incompletely understood. In this study, we evaluated changes of leukocyte superoxide dismutase (SOD) activity and plasma total nitrite as an indicator of nitric oxide (NO) levels during brucellosis therapy.

Methods: Thirty-two patients with acute brucellosis, 27 patients with chronic brucellosis and 30 healthy controls were included in the study. Patients with acute brucellosis were tested for leukocyte SOD activity and plasma total nitrite levels before, during (21st day), and at the end (45th day) of the combined therapy of rifampicin and doxycycline. The same parameters were also investigated in chronic cases and controls.

Results: The SOD activities were lower in patients with acute brucellosis before therapy compared with those 21 and 45 days after starting therapy ($P < 0.001$). In contrast, total nitrite levels did not change significantly ($P > 0.05$).

Conclusions: In the present study, leukocyte SOD activity was found to be decreased in patients with acute brucellosis. Enzyme activity was increased by treatment, finally reaching the activity of healthy controls. Using an antioxidant agent in addition to classical antimicrobial therapy for acute brucellosis might be a therapeutic approach.

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Background

Brucellosis is a zoonotic infection that remains an important health and economic burden in many undeveloped countries.[1] *Brucella* species are facultative intracellular pathogens which survive within a variety of cells, including macrophages, and the virulence of these species and the establishment of chronic infections by them are thought to be due to their ability to avoid the killing mechanism within macrophages.[2] *Brucella* infection has some analogies to infections with other intracellular pathogens such as *Listeria* and *Yersinia*. The role of macrophages in resistance to these intracellular pathogens has been described.[2] The influencing factors in *Brucella* infection appear to be more complex than in other cases and may include the production of adenine and guanine monophosphate, which suppresses the myeloperoxide-H_2O_2-halide system, and a Cu–Zn superoxide dismutase (SOD), which eliminates reactive oxygen intermediates.[1] Evidence of nitric oxide (NO)-dependent antimicrobial activity by human macrophages against parasites, fungi, bacteria and viruses is also now available.

In this study, we investigated the effect of the classical therapy regimen on leukocyte SOD activity and plasma NO concentration in acute brucellosis cases. Moreover, we compared the results with those of treatment-naive chronic cases of brucellosis and healthy controls.

Subjects and methods

This study was carried out in the Infectious Diseases and Clinical Microbiology Department of the School of Medicine of Inonu University during a two-year study

Table 1. *Clinical and laboratory findings of the patients with acute brucellosis, untreated patients with chronic brucellosis, and the controls*

	Acute (*n*=32)	Chronic (*n*=27)	Control (*n*=30)
Age median	32 ± 7.5	35 ± 8.2	31 ± 8.9
Male/female patients	17/15	14/13	14/16
Clinical findings			
Fever	32	9	0
Arthralgia	30	27	0
Night sweat	32	25	0
Hepatosplenomegaly	18	11	0
Laboratory findings			
Leukocytosis ($n > 10{,}000/mm^3$, mean ISD)	20 (12.10 ± 1.34 SD)	11 (11.15 ± 1.20 SD)	0
Leukopenia ($n < 4000/mm^3$, mean ISD)	8 (3200 ± 250 SD)	14 (2900 ± 1050 SD)	0
Normal	4	2	30
ESR>50 mL/h	30	27	0
Blood culture			
B. abortus +	20	5	0
B. melitensis +	12	4	0
Bone marrow culture			
B. abortus +	0	4	0
B. melitensis +	0	2	0
Standard agglutination test			
>1/160	18	22	0
CRP positivity (>0.6 mg/dL)	32	17	0
Rheumatoid factor negative (*n*)	32	27	30
Excluded from study	2	0	0

period. Thirty-two patients with acute brucellosis, 27 untreated patients with chronic brucellosis and 30 healthy controls were included (Table 1). There was no statistically significant difference in age or gender between the three groups ($P > 0.05$).

Patients were diagnosed by history, clinical findings and laboratory tests, including leukocyte count, erythrocyte sedimentation rate, blood and/or bone marrow cultures, C-reactive protein (CRP; CRP Latex reagent cromatest, Linear Chemicals, SL, Barcelona, Spain), standard tube agglutination test (*Brucella* tube agglutination test, Veterinary Control and Research Institute, Pendik, Istanbul, Turkey), and radiological findings. The disease was categorized into acute and chronic brucellosis according to the length and severity of symptoms.[1] Patients who had a history of brucellar spondylitis or persisting deep foci of infection for at least one year and had not received any antibrucellar therapy were considered to have chronic brucellosis. The control subjects were non-smokers, not receiving antioxidant therapy, not drinking alcohol, and had normal findings on physical and laboratory examination. They had no chronic diseases (including diabetes mellitus, hypertension and rheumatoid disorders). Pediatric brucellosis, neurobrucellosis, brucella endocarditis and pericarditis were not included in the study.

The patients and controls were informed about the purpose of the study and written consent was obtained. Blood samples were obtained after an 8 h fast for leukocyte SOD activity and plasma total nitrite measurement. Patients with acute brucellosis received rifampicin (600 mg daily, single dose, orally) plus doxycycline (200 mg twice a day, orally) for 45 days. On the 21st and 45th days of the therapy, patients were reevaluated clinically and biochemically. Two patients were excluded: one who did not respond to the therapy and one who could not tolerate the therapy.

Reagents

All solutions were prepared with distilled–deionized water. Chemicals were purchased from either Sigma

Chemical Co. (Germany), Merck Co. (Germany), or Fluka (Chemische Fabrik AG, Bushs, Switzerland).

Preparation of leukocyte sample

Leukocytes were isolated as previously described.[3] Anticoagulated venous blood (10 mL) was layered onto 2 mL of Histopaque-1077 and centrifuged at 400 *g* for exactly 30 min at room temperature. After centrifugation, plasma was saved. Leukocytes were carefully aspirated with a Pasteur pipet. Plasma and leukocytes were stored at -80°C for two months. Before measuring enzyme activities, leukocytes were lysed.[3]

Biochemical analyses

The protein concentration of leukocyte lysate was determined by the Lowry *et al.* method using serial dilutions of bovine serum albumin as standard.[4] Leukocyte SOD (E.C.1.15.1.1) activity was measured as previously described[5] and expressed as unit/mg of leukocyte protein (U/mg protein). Plasma total nitrite levels were assayed by a modification of the cadmium-reduction method[6] and results expressed as μmol/L plasma.

Statistical analysis

The χ^2 test and Student's *t*-test were used for statistical analysis using Statistical Package for the Social Sciences (SPSS).

Results

Leukocyte SOD activities were 55.8 ± 1.4 U/mg protein, 67.6 ± 2.6 U/mg protein, and 82.5 ± 1.9 U/mg protein in acute brucellosis before and on the 21st and 45th days of the therapy, respectively ($P < 0.001$ compared with pretreatment in both cases). SOD activities in untreated patients with chronic brucellosis and healthy control subjects were 50.8 ± 2.3 U/mg protein and 80.0 ± 3.9 U/mg protein, respectively. SOD activity at the end of the therapy was similar to that of the controls ($P > 0.05$).

Plasma total nitrite levels were 38.1 ± 1.1 μmol/L, 36.6 ± 3.7 μmol/L, and 34.5 ± 3.9 μmol/L in patients with acute brucellosis before and on the 21st and 45th days of therapy, respectively. Total nitrite levels were 37.0 ± 2.2 μmol/L and 33.1 ± 4.0 μmol/L in the untreated patients with chronic brucellosis and the controls, respectively. There were no significant differences in nitrite concentrations between groups.

Discussion

Brucellae ingested by phagocytes can continue to survive and replicate. Intracellular survival within macrophages is facilitated by the inhibition of phagosome–lysosome fusion by soluble products of *Brucellae* and the production of a number of stress-induced proteins. Neutrophils effectively utilize the myeloperoxidase–hydrogen peroxide–halide system to kill *Brucella*. Upon infection, these phagocytes suddenly increase oxygen consumption and produce oxygen intermediates, such as H_2O_2, O_2^-, hypochloric acid, hydroxyl radical, and single oxygen. *Brucella* species can survive in phagocytes by avoiding this system with inhibition of SOD expression.

In our study, SOD activities were lower in patients with acute brucellosis before therapy compared with patients after 21 and 45 days of therapy. In addition, SOD activity in untreated patients was similar to the pretreatment activities of acute cases and the SOD activity after treatment was similar to that of controls.

This study suggests that leukocyte SOD activity increases during treatment of patients with acute brucellosis. The levels in treated cases were similar to healthy controls. Therapy with agents which induce SOD activity in leukocytes may shorten treatment duration. In future studies, induction of SOD production by antioxidant agents or by modifying the gene structure of bacteria may add more to the treatment of the disease and may prevent chronicity.

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