Medicina (Kaunas) 2012;48(11):559-62

Tear Nitric Oxide Levels in Behçet's Disease

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Key Words: *Behçet's disease; nitric oxide; uveitis.*

Summary. *The aim of this study was to evaluate the tear nitric oxide (NO) level in patients with Behçet's disease and to compare it with that in healthy subjects.*

Material and Methods. The subjects were divided into 3 groups: the active disease, inactive disease, and control groups. NO concentrations were determined by a nitrate/nitrite colorimetric assay kit and measured spectrophotometrically at 540 nm.

Results. The tear nitrate/nitrite levels were 0.06 nmol/μL (SD, 0.05) in the active disease group, 0.05 nmol/μL (SD, 0.05) in the inactive disease group, and 0.02 nmol/μL (SD, 0.01) in the control group. The tear nitrate/nitrite levels of both active and inactive groups were higher than those of the control group (P=0.001 and P=0.047, respectively), but there was no significant difference between the active and inactive groups.

Conclusion. Our results demonstrated that the tear NO levels were elevated in the patients with Behçet's disease. We imply that a better understanding of NO function in the pathogenesis of Behçet uveitis is necessary to develop new therapies based on the inhibitors of NO synthases.

Introduction

Behçet's disease (BD) is a chronic, multisystemic vasculitic disorder with a higher prevalence being in the populations derived from the countries of the ancient Silk Road region, such as Turkey, the Middle East, and Japan (1, 2). Although the etiology remains unknown, genetic, environmental, viral, bacterial, and immunologic factors have been suggested (3). The endothelium seems to be the primary target in BD (4). Common manifestations include oral aphthous ulcers, genital ulcers, skin lesions, and uveitis. Blindness, neurologic or gastrointestinal tract alterations, arterial aneurysms, and venous thromboses are the most serious manifestations. The ocular manifestations of BD are panuveitis and vasculitis, which develop in 70% to 85% of patients, and the outcome of the inflammation is usually sight threatening (5, 6).

Nitric oxide (NO) is an important mediator of homeostatic processes in the eyes, such as regulation of aqueous humor dynamics, retinal neurotransmission, and phototransduction. Changes in its production or action could contribute to pathological conditions including inflammatory disorders such as uveitis, retinitis, or degenerative eye diseases (7). In this regard, some experimental studies suggest that NO may play a role in the pathogenesis of endotoxin-induced uveitis (EIU) as a proinflammatory mediator and that increased NO production may lead to hemodynamic and vascular permeability changes (8–13). Although several studies (14–16) have investigated the blood NO levels in patients with BD, to our knowledge, there is only one study (17) that assessed the tear NO levels of those patients. Therefore, the aim of our study was to evaluate the tear nitrate/nitrite levels as NO end products in patients with BD in comparison with those of healthy subjects.

Material and Methods

This study was carried out in the Ophthalmology Departments of Bakırköy Training and Research Hospital and Cerrahpaşa Medical School, Istanbul University. BD was diagnosed according to the criteria set by the International Study Group for BD, which consist of a required component of recurrent oral ulceration in addition to at least other 2 findings: recurrent genital ulceration, ocular inflammation, skin lesion, and positive results of the pathergy test (18). The subjects were divided into 3 groups: the active BD group, inactive BD group, and control group (healthy subjects). Since there were no clinically approved scoring system and laboratory screening profile to define the severity of Behçet's disease, both clinical and laboratory findings were used to classify the patients as having an active or an inactive disease. In clinical evaluation, patients

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having at least 3 of the major findings (skin lesions, positive results of pathergy test, uveitis, oral aphthae, and genital ulcers) were considered as having an active stage of the disease. In laboratory investigations, biochemical parameters such as an erythrocyte sedimentation rate (ESR) and a neutrophil count were investigated. The patients with BD who had 3 or more major findings and increased biochemical parameters were enrolled in the active disease group. Informed written consent was obtained from all subjects. The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee.

A standard ophthalmological examination including the determination of refractive error, visual acuity, slit-lamp examination, Goldmann applanation tonometry, and funduscopy was performed for all subjects. Fluorescein angiography was performed for all patients in order to investigate the signs of uveitis.

Subjects who had scleritis, episcleritis, ocular infection, any other systemic disorder, and a previous history of ophthalmic surgery or ocular trauma were excluded from the study.

Tear fluid was collected from the lower cul-de-sac with a micropipette by the same ophthalmologist (Y.İ.), and it was immediately frozen and stored at –20°C until analysis. For each subject, at least 85 μL of tear fluid was obtained from only one eye. The biochemists were blinded to the subjects' clinical data. NO concentrations were determined by a nitrate/nitrite colorimetric assay kit (Cayman Chemical, USA) and measured spectrophotometrically at 540 nm.

Statistical Analysis. All results were analyzed using the Number Cruncher Statistical System (NCSS) 2007 and the PASS 2008 Statistical Software program (Utah, USA). The categorical variables were compared using the chi-square test. All variables were checked with the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used for the comparison of 3 groups, and the Mann-Whitney *U* test was used to perform pair-wise comparisons. Statistical significance was set at a $P<0.05$ level.

Results

There were 16 patients (12 men and 4 women) with a mean age of 32.43 years (SD, 8.66) in the active disease group, 21 patients (13 men and 8 women) with a mean age of 32.00 years (SD, 6.65) in the inactive disease group, and 20 healthy subjects (11 men and 9 women) with a mean age of 32.18 years (SD, 7.47) in the control group. The active disease group included 9 patients with panuveitis, 4 patients with posterior uveitis, and 3 patients with anterior uveitis. There were no significant age and gender differences comparing the groups.

The biochemical parameters in the active and inactive disease groups differed significantly from the control group (Table 1). Table 2 shows the medications used by patients. There was no significant difference in the medication use between the active and inactive disease groups (all $P>0.05$).

Table 1. The Biochemical Parameters of the Subjects

Characteristic	Active Disease Group (n=16)	Inactive Disease Group (n=21)	Control Group (n=20)
ESR, mm/h	34.9 (11.6)*†	18.2 (6.6)†	10.5 (6.8)
Neutrophil count, $\times 10^3$/mL	6.0 (1.4)*†	3.6 (0.7)†	2.8 (0.7)

Values are mean (standard deviation).
ESR; erythrocyte sedimentation rate.
*$P<0.05$ compared with the inactive disease group;
†$P<0.05$ compared with the control group.

Table 2. Systemic Medication of Patients

Medication	Active Disease Group (n=16)	Inactive Disease Group (n=21)	*P* Value
Steroid	8 (50)	5 (23.8)	0.098
Azathioprine	12 (75)	11 (52.4)	0.160
Colchicine	3 (18.8)	4 (19)	0.982
Cyclosporine	6 (37.5)	3 (14.3)	0.103

Values are number (percentage).

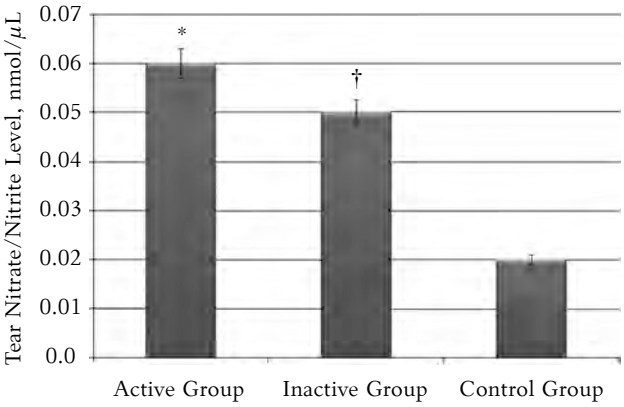

Fig. Tear nitrate/nitrite levels by groups
*$P=0.001$ as compared with the control group;
†$P=0.047$ as compared with the control group.

The tear nitrate/nitrite level was 0.06 nmol/μL (SD, 0.05) in the active group, 0.05 nmol/μL (SD, 0.05) in the inactive disease group, and 0.02 nmol/μL (SD, 0.01) in the control group. There was a significant difference in the tear nitrate/nitrite levels comparing the active and inactive disease groups with the control group ($P=0.001$, $P=0.047$, respectively); however, the active and inactive disease groups were similar in this regard (Fig.).

Discussion

Our results demonstrated that the tear NO levels of BD patients were significantly higher than those of healthy subjects. Although the tear NO level of patients with active BD was higher than those of pa-

tients with inactive BD, the difference did not reach a statistical significance.

NO has pro- and anti-inflammatory effects depending on its concentration and the site where it is released (19). It leads to endothelial vasorelaxation and the inhibition of platelet adhesion. In the presence of an excessive amount of superoxide anions and NO, peroxynitrite, a very potent oxidant, forms spontaneously (20). NO is produced by 3 isoforms of specific nitric oxide synthases (NOS): neuronal, endothelial, and inducible (iNOS) (21). In BD, T-cell lymphocytes and monocytes produce the increased levels of some cytokines (i.e., interferon γ, interleukin 2, tumor necrosis factor α) that induce NO production via the expression of iNOS (7, 22–26). It was demonstrated that the activity of iNOS was increased in EIU (9) and that the inflammatory response was reduced when the activity of NOS was inhibited (8, 9).

Evereklioglu et al. (14) demonstrated that patients with active or inactive BD had higher urinary and serum NO levels than controls. They also showed that urinary and serum NO levels were significantly higher in the patients with active Behçet's disease than those with inactive Behçet's disease. In other study (15), the serum nitrate and nitrite levels were found to be higher in the active periods of BD patients compared with the healthy controls. The authors concluded that an increased NO production in patients with BD might have critical biological activities relevant to vasculitic events in the active period of the disease. A study by Taysi et al. (16) showed an increase in NOS activity and NO levels in erythrocytes of patients with active BD as compared with those of controls, and they suggested that these parameters might play a primary role in the inflammatory reactions observed in BD. On the other hand, to the best of our knowledge, there are only 2 studies that evaluated the ocular NO levels. A study by Yilmaz et al. demonstrated that the patients who received the treatment of Behçet uveitis had the elevated levels of NO in the aqueous humor (21). A study by Mirza et al. with a small number of patients with BD (6 patients) showed that the tear nitrate/nitrite levels were decreased in the active group (6 patients) (17). However, our study demonstrated opposite results, and we think that the sample size of that study was quite small when compared with that of our study.

In our study, 8 patients (50%) in the active disease group and 5 patients (23.8%) in the inactive disease group used steroids. The difference in the steroid usage between the two patients' groups showed a trend for statistical significance. However, these patients' groups were similar concerning the tear nitrate/nitrite levels. Therefore, we do not believe that our results might be influenced by steroid use.

Conclusions

Our results demonstrated that the patients with both active and inactive BD had the elevated tear NO levels. We imply that a better understanding of NO function in the pathogenesis of Behçet uveitis is necessary to develop new therapies based on the inhibitors of NO synthetases.

Statement of Conflict of Interest

The authors state no conflict of interest.

References

1. Onder M, Gürer MA. The multiple faces of Behçet's disease and its aetiological factors. J Eur Acad Dermatol Venereol 2001;15:126-36.
2. Hirohata T, Kuratsune M, Nomura A, Jimi S. Prevalence of Behçet's syndrome in Hawaii. With particular reference to the comparison of the Japanese in Hawaii and Japan. Hawaii Med J 1975;34:244-6.
3. Onder M, Gürer MA. Behçet's disease: an enigmatic vasculitis. Clin Dermatol 1999;17:571-6.
4. Kalayciyan A, Zouboulis C. An update on Behçet's disease. J Eur Acad Dermatol Venereol 2007;21:1-10.
5. Yilmaz G, Akova Y, Aydin P. Macular ischaemia in Behçet's disease. Eye (Lond) 2000;14:717-20.
6. Akova YA, Yilmaz G, Aydin P. Retinal tears associated with panuveitis and Behçet's disease. Ophthalmic Surg Lasers 1999;30:762-5.
7. Becquet F, Courtois Y, Goureau O. Nitric oxide in the eye: multifaceted roles and diverse outcomes. Surv Ophthalmol 1997;42:71-82.
8. Mandai M, Yoshimura N, Yoshida M, Iwaki M, Honda Y. The role of nitric oxide synthase in endotoxin-induced uveitis: effects of NG-nitro L-arginine. Invest Ophthalmol Vis Sci 1994;35:3673-80.
9. Parks DJ, Cheung MK, Chan CC, Roberge FG. The role of nitric oxide in uveitis. Arch Ophthalmol 1994;112:544-6.
10. Tilton RG, Chang K, Corbett JA, Misko TP, Currie MG, Bora NS, et al. Endotoxin-induced uveitis in the rat is attenuated by inhibition of nitric oxide production. Invest Ophthalmol Vis Sci 1994;35:3278-88.
11. Goureau O, Bellot J, Thillaye B, Courtois Y, de Kozak Y. Increased nitric oxide production in endotoxin-induced uveitis. Reduction of uveitis by an inhibitor of nitric oxide synthase. J Immunol 1995;154:6518-23.
12. Jacquemin E, de Kozak Y, Thillaye B, Courtois Y, Goureau O. Expression of inducible nitric oxide synthase in the eye from endotoxin-induced uveitis rats. Invest Ophthalmol Vis Sci 1996;37:1187-96.
13. Allen JB, McGahan MC, Ferrell JB, Adler KB, Fleisher LN. Nitric oxide synthase inhibitors exert differential time-dependent effects on LPS-induced uveitis. Exp Eye Res 1996;62:21-8.
14. Evereklioglu C, Ozbek E, Cekmen M, Mehmet N, Duygulu F, Ozkiris A, et al. Urinary nitric oxide levels are increased and correlated with plasma concentrations in patients with Behçet's disease: is it a new urinary activity marker? Nephrology (Carlton) 2003;8:231-8.
15. Sancak B, Onder M, Oztas MO, Bukan N, Gürer MA. Nitric oxide levels in Behçet's disease. J Eur Acad Dermatol Venereol 2003;17:7-9.
16. Taysi S, Sari RA, Dursun H, Yilmaz A, Keles M, Cayir K,

et al. Evaluation of nitric oxide synthase activity, nitric oxide, and homocysteine levels in patients with active Behcet's disease. Clin Rheumatol 2008;27:1529-34.
17. Mirza GE, Karaküçük S, Er M, Güngörmüş N, Karaküçük I, Saraymen R. Tear nitrite and nitrate levels as nitric oxide end products in patients with Behçet's disease and non-Behçet's uveitis. Ophthalmic Res 2001;33:48-51.
18. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990;335:1078-80.
19. Stichtenoth DO, Frölich JC. Nitric oxide and inflammatory joint diseases. Br J Rheumatol 1998;37:246-57.
20. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996;271:1424-37.
21. Yilmaz G, Sizmaz S, Yilmaz ED, Duman S, Aydın P. Aqueous humor nitric oxide levels in patients with Behçet disease. Retina 2002;22:330-5.
22. Sugi-Ikai N, Nakazawa M, Nakamura S, Ohno S, Minami M. Increased frequencies of interleukin-2- and interferon-gamma-producing T cells in patients with active Behçet's disease. Invest Ophthalmol Vis Sci 1998;39:996-1004.
23. Kaneko S, Suzuki N, Yamashita N, Nagafuchi H, Nakajima T, Wakisaka S, et al. Characterization of T cells specific for an epitope of human 60-kD heat shock protein (hsp) in patients with Behcet's disease (BD) in Japan. Clin Exp Immunol 1997;108:204-12.
24. Mege JL, Dilsen N, Sanguedolce V, Gul A, Bongrand P, Roux H, et al. Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. J Rheumatol 1993;20:1544-9.
25. Goureau O, Hicks D, Courtois Y, De Kozak Y. Induction and regulation of nitric oxide synthase in retinal Müller glial cells. J Neurochem 1994;63:310-7.
26. Goureau O, Hicks D, Courtois Y. Human retina pigment epithelial cells produce nitric oxide in response to cytokines. Biochem Biophys Res Commun 1994;198:120-6.

Received 18 May 2012, accepted 30 November 2012