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Clinical and immunological effects of azithromycin in Behçet's disease

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BACKGROUND: The aim of this study was to evaluate the effects of azithromycin on mucocutaneous manifestations, oral health and immune response in Behçet's disease (BD).

METHODS: Eight BD patients with active mucocutaneous symptoms were treated with azithromycin for 4 weeks. Oral health, clinical manifestations and *in vitro* interleukin (IL)-12, interferon (IFN)-γ, IL-10 and monocyte chemotactic protein (MCP)-1 responses were evaluated before and after treatment.

RESULTS: The number of folliculitic lesions, healing time of oral ulcers and scores of plaque indexes (PLIs) were lower after azithromycin treatment ($P < 0.05$). Scores of PLIs correlated positively with the healing time of oral ulcers ($P = 0.02$). Although a trend towards increased stimulated IL-10 responses with azithromycin was observed, no statistically significant difference was found. Stimulated and unstimulated MCP-1, IFN-γ and IL-12 responses were similar before and after treatment ($P > 0.05$).

CONCLUSION: Azithromycin was observed to be effective in decreasing folliculitic lesions and fastening the healing time of oral ulcers in BD.

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Keywords: antimicrobial treatment; azithromycin; Behçet's disease; cytokines and chemokines

Introduction

Behçet's disease (BD) is a multisystemic disorder, characterized by oral and genital ulcers, cutaneous, ocular, arthritic, vascular and central nervous system involvement (1, 2). Although the aetiology and pathogenesis is not clearly defined, genetic predisposition (HLA-B51), infections and immunological dysfunctions have been implicated (2–4). Clinical flares were observed after dental procedures and cutaneous tests with streptococcal extracts. Various atypical streptococcal species are also documented more frequently in the oral flora of patients with BD (5–8). In addition, oral health is impaired and is associated with a more severe course, reflecting the effects of microbial flora on the pathogenesis of the disease (9). Results of limited studies examining the effects of antimicrobial treatment for BD are also promising (10, 11). Azithromycin, a macrolide with a long half-life (12), is an effective agent in both adjunctive and prophylactic treatment of chronic oral infections (13). Azithromycin also has immunomodulatory properties such as suppression of neutrophil responses and cytokine/chemokine expressions (14).

The aim of this study was to determine the efficacy of azithromycin compared with the current drug of choice, colchicine, for the treatment of mucocutaneous manifestations of BD and to examine its effects on *in vitro* cytokine and chemokine responses of peripheral blood mononuclear cells (PBMCs).

Methods

Patients and controls

Eight patients with BD (four female, four male, mean age: 28.2 ± 8.1 years, range:17–38 years) fulfilling the International Study Group Criteria (15) and followed at the Outpatient Behçet's Clinics of Marmara University Hospital, Istanbul, took part in the study. Criteria of inclusion in the study were: age between 17 and 60 years, presence of active mucocutaneous involvement defined as the occurrence of at least one episode of oral and genital ulcers, erythema nodosum or papulopustular lesions within the last month. The presence of eye disease, neuropathy, vascular involvement and any other organ involvement requiring immunosuppressive treatment, recent exposure to systemic immunosuppressive therapy for at least 3 months and severe drug reactions were the exclusion criteria. After a 4-week pretrial observation period, patients were treated by oral azithromycin (500 mg) three times a week for 4 weeks

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and assessed at the end of this period. After a 10-day washout period, patients then started to receive colchicine 1.5 mg/day for another 4 weeks. Baseline (pre-treatment), post-azithromycin and post-colchicine clinical assessments were done by the same physicians [a dentist (GM), dermatologist (TE) and a rheumatologist (HD)]. The frequency of oral and genital ulcers, their healing time, dental and periodontal health, attacks of erythema nodosum and the number of papulopustular lesions and arthritis as well as side-effects were recorded in each visit. *In vitro* cytokine and chemokine responses were also investigated. In between clinical examinations, patients filled their personal record chart, regarding the occurrence and duration of oral and genital ulcers and for attacks of erythema nodosum. The study was performed according to the principles of the Decleration of Helsinki and was approved by the Ethical Committee of Marmara University Medical School. Informed consent was taken from the patients.

Oral health

Score of plaque index (PLI), gingival index (GI), sulcus bleeding index (SBI), probing depth (PD) were recorded to evaluate oral infection foci by the same dentist (GM) in BD outpatient clinics. Recordings included all the teeth except third molars (16).

Cytokine and chemokine responses

The PBMCs of patients with BD were separated from 10 cc heparinized blood by density gradient centrifugation (Lenfoprep 1077; Sigma, Deisenhofen, Germany). Isolated PBMCs were washed in 0.9% NaCl solution in two times, were incubated as 10^5/well at 37°C, in an atmosphere of 5% CO_2 in 96 well-culture plates (Costar, Cambridge, MA, US) supplemented with RPMI-1640 medium, L-Glutamine (2 mmol/l), penicillin (100 U/ml), streptomycin (100 μg/ml) and fetal calf serum (10%). After 48 h of stimulation with *Streptococcus sanguis* (10 μg/ml, kindly provided by F. Kaneko, University of Fukushima, Japan), 100 μl of cell culture supernatants were collected and stored at −40°C. Stimulated and unstimulated interleukin (IL)-10, interferon (IFN)-γ (Diaclone, Paris, France), IL-12 and MCP-1 (Immunotech, Marseïlle, France) responses were assayed with commercial enzyme-linked immunosorbent assay kits.

Statistical analysis

Wilcoxon rank and Spearman correlation tests were used. Data was analysed in an SPSS (11.5) programme and a P-value < 0.05 was accepted as significant.

Results

Clinical results

Mean disease duration was 4.1 ± 3.2 years (range: 1–9 years) in the study group. No significant differences were observed according to gender (females: 3.5 ± 1.0; males: 4.5 ± 1.0) ($P = 0.2$). Active clinical manifestations of BD patients before azithromycin treatment (baseline clinical examination) were as follows: oral ulcers (100%, $n = 8$), genital ulcers (62.5%, $n = 5$), erythema nodosum (62.5%, $n = 5$), folliculitis (100%, $n = 8$) and arthritis (50%, $n = 4$). Positive pathergy reaction was present in 50% of the patients ($n = 4$).

The number of patients with oral and genital ulcers decreased after both treatment modalities (Fig. 1). Although the mean number of oral ulcers was lower in patients on azithromycin (3.8 ± 3.3) compared with pre-treatment (6.4 ± 4.5) and colchicine (4.2 ± 2.9), no significant difference was observed among the groups. However, the healing time of oral ulcers decreased significantly after azithromycin treatment (pre-treatment: 9.1 ± 4.8 days, azithromycin: 4.4 ± 1.5 days) ($P = 0.04$). Although colchicine also effected the healing time (6.5 ± 3.8), the difference was not statistically significant ($P = 0.17$). Follicular lesions completely healed with azithromycin in the study group. However, only colchicine was effective for erythema nodosum (two of eight) compared with pre-treatment (five of eight) and azithromycin (four of eight) (Fig. 1).

Plaque index scores were also significantly decreased with azithromycin treatment (0.5 ± 1.1) compared with pre-treatment period (1.9 ± 0.9) ($P = 0.04$). PLI scores positively correlated with the healing time of oral ulcers (4.4 ± 1.5) ($r = 0.7$, $P = 0.02$). Although a trend towards a decrease in PLI scores was also observed with colchicine (1.1 ± 1.1), the difference did not reach significance ($P = 0.06$). Although tooth brushing frequency was higher in patients after azithromycin (1.0 ± 0.1) than pre-treatment (0.7 ± 0.7) and colchicine (0.8 ± 0.8), no significant differences were observed ($P = 0.18$ and $P = 0.65$, respectively). Lack of tooth brushing was negatively correlated with score of PLI in pre-treatment and post-colchicine ($r = -0.5$ $P = 0.02$ and $r = -0.8$ $P = 0.04$, respectively).

Although scores of GI and SBI after azithromycin (1.1 ± 1.0 and 0.8 ± 1.2, respectively) also decreased compared with those before treatment (1.8 ± 0.6, 1.9 ± 0.8) and colchicine (1.5 ± 1.1, 1.7 ± 1.3), statistically significant differences were not present ($P = 0.07$ and $P = 0.09$, respectively). Similarly, PD scores were also lower in patients after azithromycin (1.9 ± 0.5) than pre-treatment (2.2 ± 0.6) and colchicine

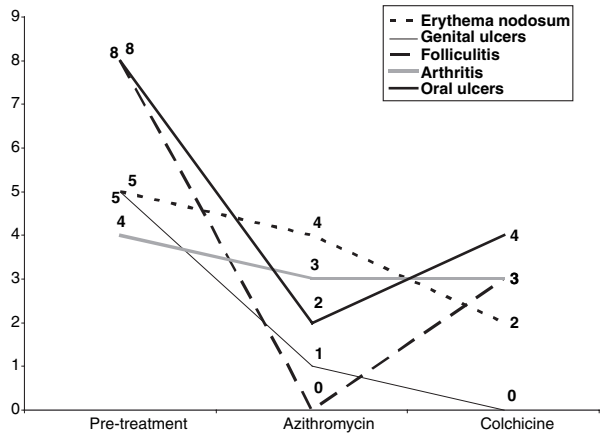
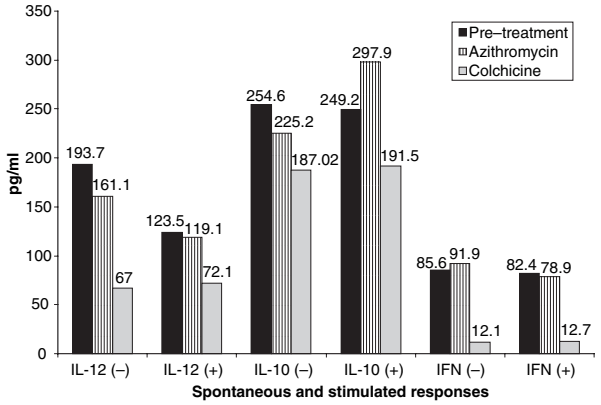


Figure 1 Clinical manifestations in patients with Behçet disease according to different treatment modalities.



(–): Spontaneous responses, (+): Stimulated responses with *S. sanguis*

Figure 2 Cytokine responses of patients with Behçet's disease according to different treatment modalities.

(2.1 ± 0.7), without significant differences ($P = 0.34$ and $P = 0.20$, respectively).

Diarrhoea was seen in two patients using azithromycin, which did not require withdrawal. No side-effect was observed during colchicine treatment.

In vitro cytokine and chemokine responses

Unstimulated and stimulated IL-12 responses with *S. sanguis* were higher in pre-treatment and with azithromycin, compared with colchicine treatment (before: $P = 0.02$ and $P = 0.04$, after: $P = 0.04$ and $P = 0.04$, respectively) (Fig. 2). Unstimulated and stimulated IFN-γ responses were similar in pre-treatment and azithromycin samples ($P = 0.61$ and $P = 0.93$, respectively). However, unstimulated IFN-γ responses were lower with colchicine compared with pre-treatment ($P = 0.046$). Although IFN-γ responses stimulated with *S. sanguis* were also higher with azithromycin than colchicine, no significant difference was observed ($P = 0.07$) (Fig. 2). Although a trend towards higher stimulated IL-10 responses with azithromycin was observed compared with other groups, statistically significant differences were not found ($P > 0.05$) (Fig. 2). MCP-1 responses were similar in pre-treatment (spontaneous: 143.3 ± 289.3; stimulated: 304.7 ± 345.9), azithromycin (146.8 ± 125.02 and 420.1 ± 446.8, respectively) and colchicine (250.6 ± 238.6 and 573.7 ± 841.6, respectively) groups.

Discussion

The etiologic relationship between streptococcal antigens and BD has been postulated in various studies (5–7). Antibiotics might have a place in the treatment spectrum of BD for the eradication of streptococcal infections that can trigger inflammatory episodes (10, 11). Effects of azithromycin, a macrolid antibiotic, on mucocutaneous manifestations and immune responses in patients with BD were investigated in the present study. Positive effects of azithromycin treatment were especially prominent on the healing time of oral ulcers and the elimination of folliculitis. Macrolids can also be used to counter oral infections as they can rapidly inhibit bacterial activity with consistent levels detected in saliva and periodontal tissues (17). Related with this phenomenon, antibiotic effect of azithromycin could decrease the duration of oral ulcers. The presence of painful oral ulcers is a significant risk factor for an increase in plaque accumulation because of the limitation of oral hygiene applications (18). The decline in plaque accumulation can be claimed to prolong the duration of ulcer free intervals. We have previously reported that plaque accumulation was higher in patients with active oral ulcers compared with ulcer-free patients with BD (9). In addition, plaque accumulation (odds ratio: 2.3) was a significant risk factor for increased clinical severity scores reflecting its association with systemic involvement (9). In this context, the elimination of oral ulcers might also have beneficial effects on oral hygiene habits.

As azithromycin is an effective treatment for acne (12), it is not unexpected that folliculitic acneiform lesions completely healed with azithromycin. However, no significant difference was observed in the number of erythema nodosum attacks after azithromycin. Çalgüneri et al. previously reported that benzathine penicillin plus colchicine was superior to colchicine alone in BD (10). Similarly, oral minocycline treatment for 3 months led to a reduction in the frequency of EN and folliculitic lesions and had a modest effect on oral ulcerations (11). In a case report, a patient with severe erythema nodosum who was refractive to classical treatments dramatically responded to erythromycin (500 mg four times daily in a 10-day period) (19).

Macrolids have been shown to possess anti-inflammatory properties independent of their antimicrobial activity (20). Macrolids may also reduce the production of pro-inflammatory cytokines such as IL-1, IL-6, tumour necrosis factor (TNF)-α and IL-8 (14, 20, 21). IL-1β and IL-6 productions in PBMCs supernatants incubated with *S. sanguis* was reduced by minocycline treatment in BD (11). We observed no significant effect of azithromycin on IL-12 and IFN-γ responses suggesting that azithromycin does not modulate Th1 type responses. However, a trend towards a favourable effect on IL-10 responses was observed, which might counter-regulate the pro-inflammatory milieu in BD sera. Alternatively, other direct effects on pro-inflammatory monocyte-macrophage derived cytokines and neutrophil functions might be associated with the anti-inflammatory effects of azithromycin in our study.

The prominent effect of colchicine was the elimination of erythema nodosum attacks with a similar trend for oral ulcer relapses. Colchicine is commonly used as the first-line treatment of mucocutaneous manifestations of BD (22, 23). It was found to be an effective agent in treating erythema nodosum in both sexes. Apthous ulcers were also significantly improved by colchicine, especially in females (23). In *in vitro* cytokine responses, stimulated responses of IL-12 and IFN-γ were significantly decreased by colchicine treatment. Overproduction of Th1 type proinflammatory cytokines is prominent especially in active BD patients (2, 3, 7).

Elevated IL-12 might activate the adaptive immune system and IL-12 levels are shown to correlate with active disease (4). In attack-free, asymptomatic FMF patients, serum levels of IL-6, IL-8, and TNF-α showed a significant decrease after 2 months of colchicine treatment (24, 25). The effects of colchicine on Th1 responses in our study might be the direct suppression of IL-12 and IFN-γ released from mononuclear cells or an indirect effect through pro-inflammatory monocyte-macrophage derived cytokines.

The main shortcoming of our study is its short-duration. However, as no previous safety or efficacy data was present for azithromycin in BD, we decided to perform a short, preliminary study with a limited number of patients. Still our results with azithromycin are encouraging with prominent effects on oral ulcer healing and folliculitis and a good safety profile. The combination of azithromycin with colchicine might also have a synergistic effect on different mucocutaneous manifestations of BD, as they seem to modulate different cytokines. Further controlled studies are planned for a definitive assessment of the efficacy and safety of azithromycin in the management of BD.

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