Comparison of ultraviolet B-induced cutaneous inflammation and skin pathergy test in Behçet's disease

Background: Nonspecific hyper-reactive inflammatory response is an important feature of Behçet's disease (BD), but the underlying mechanism has yet to be identified. Ultraviolet B (UVB)-induced erythema is a well-established experimental method for the investigation of antigen-independent cutaneous inflammation, and no data are available on UVB response in BD patients. **Aim:** To investigate the UVB-induced erythema by determining the minimal erythema dose in BD patients and matched healthy controls, and also to compare the results of UVB response with the skin pathergy reaction. **Patients & methods:** The study group consisted of 47 patients with BD, 32 with various rheumatic diseases and 21 healthy volunteers. UVM-57 (Ultraviolet Products, Cambridge, UK; 302 nm), was used as the narrow-band UVB source. The forearm of each individual was exposed to a 0.14–10.45 J/cm^2 dose of 302-nm UVB with 0.045 J/cm^2 increments. The skin pathergy reaction was tested using a 20 G hypodermic needle as previously described. The erythema and skin pathergy reaction were observed at 24 and 48 h. **Results:** The mean minimal erythema dose and minimal erythema dose duration in BD patients was found to be lower than those in controls, and the difference was statistically significant between BD patients and diseased controls at 48 h (0.27 ± 0.08 vs 0.32 ± 0.07 J/cm^2; and 2.95 ± 0.92 vs 3.5 ± 0.07 min, respectively; p = 0.032 for both), and between BD and combined (diseased plus healthy) control groups at 24 h (0.24 ± 0.09 vs 0.29 ± 0.07 J/cm^2 and 2.76 ± 0.82 vs 3.18 ± 0.75 min, respectively; p = 0.036 and p = 0.05, respectively) and 48 h (0.27 ± 0.08 vs 0.31 ± 0.07 J/cm^2 and 2.95 ± 0.92 vs 3.38 ± 0.75 min, respectively; p = 0.039 for both). This difference becomes more prominent when patients and controls with the same skin color (type 3) were compared. **Conclusion:** The development of a cutaneous erythematous response with significantly lower doses of UVB further supports the nonspecific hyper-reactive inflammatory characteristics of BD and suggests that it can be induced without any antigen. These preliminary results may help in understanding the cutaneous inflammation in BD and developing alternative methods to investigate it.

KEYWORDS: Behçet's disease ■ cutaneous inflammation ■ skin pathergy test ■ ultraviolet B

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Behçet's disease (BD) is a chronic, relapsing multisystem inflammatory disorder of unknown etiology, presenting with mucocutaneous, ocular, vascular, articular, intestinal and neurological manifestations. Mucocutaneous lesions include recurrent oral aphthae, genital ulceration, acne form lesions, erythema nodosum-like lesions and skin pathergy reaction (SPR). The SPR is a nonspecific hyper-reactivity of skin to the needle prick; and it has been included as one of the five criteria for the classification of BD by the International Study Group (ISG) [1]. Although the prevalence of a positive SPR varies in different countries depending on many variables, a positive skin pathergy test (SPT) has been suggested to be highly specific for BD [2–4].

The SPR is characterized by an erythematous papule or a sterile pustule formation at 48 h following an intradermal needle prick, but the pathogenesis of skin hyper-reactivity has not been well understood. Histopathological examination of SPR suggests an antigen-independent inflammatory response with a mixed cellular perivascular infiltrate [5]. In most studies, the SPR was induced by either a solo needle prick or by intradermal injection of physiological saline [6]. It has also been shown that the surgical cleaning of the forearm skin surface with disinfectants for 4 min before the application of the needle reduced the prevalence of SPR positivity, suggesting that some substances, bacteria or skin products eliminated by the surgical cleaning may have played a role in the development of SPR [7]. On the other hand, we previously demonstrated that the physical trauma of argon laser photocoagulation could also induce a pathergy-like skin inflammation in BD patients with its thermal injury and without inoculation of any antigen with needles [8].

Ultraviolet (UV) B (UVB) irradiation has been known to cause biological reactions in the skin, inducing an inflammatory response and

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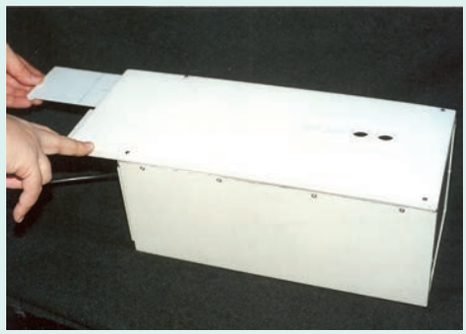

Figure 1. Ultraviolet B applications box.

apoptosis, subsequently leading to skin damage. The inflammatory changes of acute exposure to UVB of the skin include erythema, production of inflammatory mediators, alteration of vascular responses and infiltration of inflammatory cells [9].

In this study, we aimed to investigate the UVB-induced erythema in patients with BD and healthy controls as a model of cutaneous inflammation along with the SPR induced by hypodermic needle pricks to further understand the underlying mechanisms of hyper-reactive inflammatory response in BD.

Patients & methods

The study group consisted of 47 patients with BD (28 males and 19 females), 21 healthy controls (ten males and 11 females) and 32 diseased controls with various rheumatic diseases (eight males and 24 females). All BD patients fulfilled the ISG classification criteria [1]. Healthy controls described no inflammatory or skin-related disorders. For the diseased control group, patients with clinical findings related to BD and certain diseases with an SPR-like skin hyper-reactivity, such as pyoderma gangrenosum, erythema elevatum diutinum and Sweet's syndrome, were excluded. Thirty two patients who were followed-up at the Division of Rheumatology (Istanbul Faculty of Medicine in Istanbul University, Istanbul, Turkey; nine with rheumatoid arthritis, six with systemic lupus erythematosus, five with systemic vasculitis, four with systemic sclerosis, three with psoriatic arthritis, two with seronegative arthritis, one with mixed connective tissue disease, one with osteomalacia and one with post-streptococcal reactive arthritis) were enrolled into the study. The ethics committee approved the study protocol.

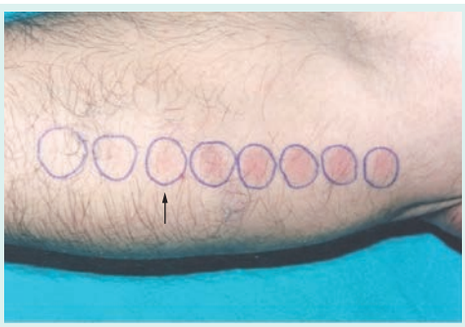

Figure 2. Clinical appearances of ultraviolet B-induced erythema at 48 h. Arrow demonstrates perceptible erythema (minimal erythema dose).

Since response to UVB irradiation depends on skin color, all participants were classified according to the Fitzpatrick system [10], and only those with type 3 and type 4 skin were enrolled in the study. Relatively less hairy and avascular regions in the flexor parts of both forearms were selected as test regions, and both arms of the participants were cleaned with ethyl alcohol before the investigative procedures.

UV device

A portable 6 W, UVM-57 model device produced by Ultraviolet Products (Cambridge, UK) was used as a narrow-band UVB source, which had the property of producing UV radiation at one wavelength (302 nm). The power of UV source (relative density) was 1500 pW/cm^2 when used at a distance of 3 inches (~7.6 cm).

UV application box

A box was developed for investigation purposes to standardize the UV radiation. The UVB device was located at the base of a quadrangular prism box, radiating part standing upwards. The upper surface of the box, which was 7.6 cm above the UV source, and onto which the forearm should be placed, was divided into two parts. Eight sequenced holes with a 13-mm diameter were formed on one part, and four sequenced holes with a 21-mm diameter on the other. These holes could be opened in order by a sliding cover (Figure 1). By increasing the number of open holes by the sliding cover, we managed to give different doses of UVB to different holes, maximum to the first opened hole, and minimum to the last one.

Application of UVB

UVB was applied first to the right, then to the left arms of the individuals. The ventral part of the right forearm was located on the site with eight holes, covering all of them completely. Starting from the first hole, all holes were opened with 30 s intervals, by sliding the cover.

UVB was applied to the last hole for 1.5 min. By this method, the first hole received UVB for 5 min, and the other holes for 4.5, 4, 3.5, 3, 2.5, 2 and 1.5 min, in order (Figure 2). After this, the left forearm was located on the site with four holes with greater diameters. Starting from the first hole, all holes were opened with 1-min intervals. UVB was applied to the last hole for 2 min. By this way, first hole received UVB for 5 min and the last hole for 2 min. The borders of UVB-applied surfaces were drawn with pen. All patients and controls were advised to protect their arms from sunlight with long-armed clothes and not to bathe for 2 days.

Skin pathergy test

The left forearm of all the study groups were cleaned with ethyl alcohol before the procedure. Relatively less hairy and avascular regions in the flexor part of the left forearm were selected as test regions. The SPR was tested with three pricks performed using a 20 G hypodermic needle. In addition, in order to understand whether the SPR reaction is influenced by UVB application or not, needle pricks were also applied to the sites of first and the second radiations (5 and 4 min) on left forearm (Figure 3).

Identification of minimal erythema dose

Minimal erythema dose (MED) was described as the minimal dose of UVB producing perceptible erythema at 24 h on the forearm at UVB application sites. The first hole at which erythema was determined (Figure 2) and the UV dose was calculated from the period of UVB application as described in Table 1.

Statistical analysis

MED and MED durations determined in patients with BD and controls were compared by the nonparametric Mann–Whitney U test. In addition, MED values of patients with BD were compared similarly, according to whether they had positive or negative SPR results.

Results

Demographic and clinical features of the study population are shown in Table 2. An erythematous skin reaction was not observed in 16 out of 47 (34%) patients with BD, six out of 21 (29%) healthy controls (with only type 3 and type 4 skin) and 13 out of 32 (41%) diseased controls within the tested UVB exposure ranges, and there was no statistical difference between the groups (p = 0.66). Individuals without erythema after UVB application were excluded from the study for statistical evaluation. Characteristics of patients with BD and the controls who had erythema formation after UVB application are shown in Table 3.

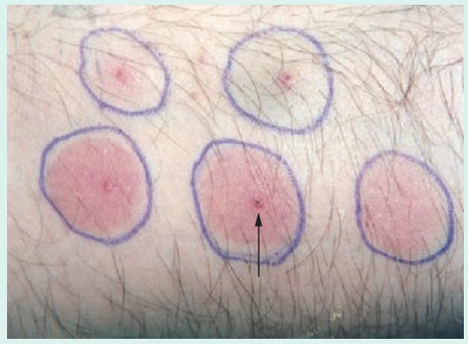

Figure 3. Clinical appearances of positive skin pathergy test at the region of ultraviolet B-induced erythema at 48 h on left forearm (arrow).

The mean MED measurements (J/cm^2) and MED durations (min) were not found to be significant in BD patients compared with the measurements obtained in diseased and healthy controls at 24 h. These differences were statistically significant when BD patients were compared with the combined control group (p = 0.036 for MED and p = 0.05 for MED duration) at 24 h BD; and with the diseased and combined controls groups at 48 h (p = 0.032 for MED and MED durations in diseased controls and p = 0.039 for MED and MED durations in the combined control group; Table 4).

In order to prevent the error due to the difference in skin color types, we also assessed the

Table 1. Ultraviolet B doses corresponding to application periods†.

Duration (min)	Dose (J/cm^2)
0.5	0.045
1	0.09
1.5	0.14
2	0.18
2.5	0.23
3	0.27
3.5	0.32
4	0.36
4.5	0.41
5	0.45

†Calculated according to the fact that the UVM-57 device was producing radiation at a density of 1500 µW/cm^2 from a distance of 7.6 cm.

Table 2. Demographic and clinical features of the study groups.

Characteristics	Behçet's disease patients (n = 47)	Healthy controls (n = 21)	Diseased controls (n = 32)
Male/female	28/19	10/11	8/24
Mean age ± standard deviation (range), years	36.4 ± 10.2 (20–58)	24.85 ± 3 (21–33)	43 ± 13.5 (17–66)
Skin type 3 (n)	41	16	28
Skin type 4 (n)	6	5	4
Duration of disease (years) ± standard deviation (range)	8.1 ± 6 (0.5–23)		
Oral ulcers, n (%)	47 (100)		
Genital ulcers, n (%)	29 (62)		
Skin manifestations, n (%)	43 (91)		
Ocular involvement, n (%)	17 (36)		
Arthritis, n (%)	24 (51)		20 (62.5)
Superficial thrombophlebitis, n (%)	12 (25.5)		
CNS involvement, n (%)	2 (4)		
Deep vein thrombosis, n (%)	5 (10.6)		
Pulmonary arterial aneurysm, n (%)	5 (10.6)		

results of only the participants with type 3 skins (n = 56). In patients with BD (n = 27), MED and MED duration measurements were significantly lower than diseased controls (n = 16) both at 24 and 48 h (p = 0.035 for MED and p = 0.044 for MED duration; and p=0.021 for MED and p=0.021 for MED duration, respectively), but the difference did not reach a statistically significant level during the comparison with healthy controls (n = 13). Similarly, the mean MED values of type 3 BD patients were found to be significantly lower than the mean of the combined control group both at 24 and 48 h (p = 0.014 and p = 0.022, and p = 0.018 and p = 0.018, respectively; Table 5).

A positive SPR was observed in 11 BD patients (32%) during the study, and in none of the control groups. We did not observe any influence of UVB irradiation on SPR positivity, and we could not find any significant difference between the mean MED values and MED durations of BD patients with or without positive SPR response (Table 6).

Discussion

Nonspecific hyper-reactivity to trauma is an important feature of BD; and SPR, which has been known since 1937, has been widely used as an important tool in the diagnosis of BD [11]. The positive SPR, a reaction very similar to erythematous indurations or pustules that appear spontaneously in patients with BD, has been suggested to be highly specific for BD, especially when it is positive at 48 h [12].

Table 3. Characteristics of patients with Behçet's disease and the controls who had erythema formation after ultraviolet B application.

Characteristics	Behçet's disease patients (n = 31)	Healthy controls (n = 15)	Diseased controls (n = 19)
Male/female	23/8	8/7	8/11
Mean age ± standard deviation (range), years	37.6 ± 9.7 (22–57)	26.1 ± 3 (23–33)	41.4 ± 14.5 (17–66)
Duration of disease (years) ± standard deviation (range)	8.13 ± 6 (0.5–23)		
Skin type 3 (n)	27	13	16
Skin type 4 (n)	4	2	3

Table 4. Results of the mean minimal erythema dose and minimal erythema dose durations in patients with Behçet's disease and control groups at 24 and 48 h.

MED values	Behçet's disease patients (n = 31)	Healthy controls (n = 15)	p-value	Diseased controls (n = 19)	p-value	Total controls (n = 34)	p-value
MED at 24 h (J/cm^2)	0.24 ± 0.09	0.28 ± 0.06	0.147	0.29 ± 0.07	0.052	0.29 ± 0.07	0.036
MED duration at 24 h (min)	2,76 ± 0.82	3.1 ± 0.69	0.194	3.24 ± 0.08	0.063	3.18 ± 0.75	0.05
MED at 48 h (J/cm^2)	0.27 ± 0.08	0.29 ± 0.07	0.242	0.32 ± 0.07	0.032	0.31 ± 0.07	0.039
MED duration 48 h (min)	2.95 ± 0.92	3.23 ± 0.78	0.242	3.5 ± 0.07	0.032	3.38 ± 0.75	0.039

MED: Minimal erythema dose.

The SPR is also a convenient model for the investigation of increased inflammatory activity, since the beginning of the inflammatory reaction can be determined, and the progression of inflammation can be monitored. The underlying mechanism of SPR has yet to be identified, and understanding of its mechanisms may help to clarify the pathogenesis of BD. It is still not clearly understood whether antigenic inoculation (e.g., micro-organism, the mechanical disruption of epidermal or dermal components) occurs while performing the SPR test, and the roles of exogenous antigens or endogenous danger signals in triggering inflammatory exacerbations of BD are still undetermined.

Dilsen *et al*. concluded that there was a positive correlation between SPR and the magnitude of the induced trauma [2]. After the introduction of the disposable/sharp needles, which are less traumatic than the nondisposable/blunt needles, the prevalence and intensity of positive SPR has been known to decrease. In addition, Gul *et al*. found that the SPR was restricted to the early phase of cutaneous inflammation, which can develop without any antigenic stimulation [13]. In a previous study, we used an argon laser photocoagulation-induced skin inflammation model to test the skin hyper-reactivity in BD patients; and we observed that the thermal skin injury laser photocoagulation produced a similar hyper-reactive pathergy response without pricking the skin by a needle [8]. In this study, we tried to test another physical trauma, which protects the skin integrity, to further understand the skin hyper-reactivity in BD by a UVB-induced skin inflammation model.

Narrow-band UV lamps are being used increasingly for phototherapy of psoriasis, vitiligo and various other dermatoses. UVB-induced inflammation enhancing epidermal hyperplasia through the release of proinflammatory cytokines, growth factors and various other inflammatory mediators, including prostaglandins, which result in erythema, alteration of vascular responses and inflammatory cell infiltrate [9,14].

Erythematous reaction could not be induced in a similar proportion of BD patients and controls with the planned UVB exposure times (34% of BD patients, 41% of diseased controls and 29% of healthy controls), which may result from various factors including skin type, exposure to sunlight and epidermal thickness [9]. Since the ratios of individuals without erythema were equal in all groups, we excluded those individuals from the study and considered that it may not cause any bias affecting the results.

When patients with BD were compared with healthy and diseased controls, the mean MED values and MED durations of patients with BD were found to be lower, suggesting a hyperreactive inflammatory response to UVB exposure. This was a pilot study to collect preliminary data on the response of BD patients to UVB, and

Table 5. Results of Behçet's disease patients and the control groups with type 3 skin.

MED values	Behçet's patients (n = 27)	Healthy controls (n = 13)	p-value	Diseased controls (n = 16)	p-value	Total controls (n = 29)	p-value
MED at 24 h (J/cm^2)	0.23 ± 0.09	0.28 ± 0.07	0.064	0.29 ± 0.08	0.035	0.29 ± 0.07	0.014
MED duration at 24 h (min)	2.65 ± 0.79	3.12 ± 0.74	0.094	3.22 ± 0.86	0.044	3.18 ± 0.80	0.022
MED at 48 h (J/cm^2)	0.26 ± 0.09	0.30 ± 0.08	0.151	0.32 ± 0.07	0.021	0.31 ± 0.07	0.018
MED duration at 48 h (min)	2.87 ± 0.94	3.27 ± 0.83	0.151	3.53 ± 0.76	0.021	3.41 ± 0.79	0.018

MED: Minimal erythema dose.

Table 6. The mean minimal erythema dose and minimal erythema dose durations according to the skin pathergy reaction results in patients with Behçet's disease.

MED values	SPR positive (n = 11)	SPR negative (n = 20)	p-value
MED at 24 h (J/cm^2)	0.26 ± 0.08	0.23 ± 0.09	0.38
MED duration at 24 h (min)	2.9 ± 0.9	2.68 ± 0.8	0.53
MED at 48 h (J/cm^2)	0.28 ± 0.09	0.26 ± 0.08	0.79
MED duration at 48 h (min)	3.05 ± 1	2.9 ± 0.9	0.79

MED: Minimal erythema dose; SPR: Skin pathergy reaction.

they lack enough power, mainly resulting from the size of the control groups as well as patients, and a larger study is expected to provide further insights on the triggering of hyper-inflammatory skin response in BD patients.

Conclusion & future perspective

SPR has been recognized as a problematic diagnostic tool with difficulties in evaluation and obtaining reproducible results. Therefore, observing no correlation between MED and SPR positivity should not suggest that they are associated with different inflammatory pathways. Since SPR is still regarded as an important tool in diagnosis of BD, we need to improve its reproducibility as well as explore the possibility of alternative tools testing the skin hyper-reactivity, similar to the studies with uric acid crystals. In this regard, the preliminary results of this pilot study warrant further research on alternative methods in the analysis of hyper-reactive inflammatory response in BD, and we hope that all these efforts would help to develop a more standardized way of testing pathergy reaction using an acceptable trigger.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Nonspecific hyper-reactive inflammatory response is an important feature of Behçet's disease (BD).
- Ultraviolet B (UVB)-induced erythema is a well-established experimental method for the investigation of antigen-independent cutaneous inflammation, and no data are available on UVB response in BD patients.
- Minimal erythema dose and minimal erythema dose duration in BD patients was found to be significantly lower than those in controls, suggesting a hyper-reactive inflammatory response to UVB exposure.
- Skin pathergy reaction has still been regarded as an important tool in the diagnosis of BD, we need to improve its reproducibility as well as to explore the possibility of alternative tools testing the skin hyper-reactivity.
- The development of a cutaneous erythematous response with significantly lower doses of UVB further supports the nonspecific hyper-reactive inflammatory characteristics of BD and suggests that it can be induced without any antigens.

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