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Raised plasma adrenomedullin level in Behçet's disease patients

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Abstract To investigate the role of plasma adrenomedullin (AM) in the pathogenesis of Behçet's disease (BD) patients with inactive ocular complications or ocular attack, 18 consecutive BD patients with ocular complications, including 1 BD patient with ocular attack, another group of 6 BD patients with ocular attack, and 10 normal volunteers were evaluated. All BD patients were regularly followed at ophthalmic outpatient clinics. Levels of both total and mature AM in plasma were measured by immunoradiometric assay. Plasma levels of endothelin-1 (ET-1) were also measured by radioimmunoassay. We also measured the levels of C-reactive protein (CRP) in plasma. Levels of total AM in plasma (mean ± SD, 19.6 ± 6.9 fmol/l) were significantly higher in BD patients than in normal volunteers (14.5 ± 3.6 fmol/l) ($P = 0.01$). The levels of mature AM were also higher in BD patients (1.6 ± 0.4 fmol/l) than in normal volunteers (0.3 ± 0.6 fmol/l) ($P = 0.002$). The levels of AM in patients with ocular attack were higher than those in normal volunteers, although there was no significant difference compared to levels of AM in BD patients without ocular attack. AM may play an important role as an antiinflammation factor or may reflect endothelial damage as a marker of disease activity in BD patients.

Key words Adrenomedullin (AM) · Behçet's disease (BD) · Endothelin-1 (ET-1) · Ocular attack

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Introduction

Behçet's disease (BD) is a polysymptomatic and recurrent systemic vasculitis with a chronic course and an unknown cause. The pathology of the lesions consists of widespread vasculitis. Eyes, skin, joints, oral cavity, blood vessels, and central nervous system are usually involved.[1] Although the pathogenesis of the disease still remains unclear, Sakane et al. reported that its cause might be (a) genetic predisposition, (b) abnormalities of the immune system such as immunoactive cells, (c) T cells infiltrated into the affected legions with activation of circulating T and B cells, followed by chemotaxis of neutrophlils or vice versa, or (d) endothelial damage.[1] In fact, it has been reported that the plasma levels of endothelin-1 (ET-1) are elevated, which may relate to thrombosis and vasculitis in BD.[2–4]

Adrenomedullin (AM) is a hypotensive peptide found in human pheochromocytoma tissue, which comprises 52 amino acids with an intramolecular disulfide bond. The ring structure and amidated C-terminus of AM are critical for its receptor binding and hypotensive activity. The mature AM is synthesized as glycine-extended AM followed by C-terminal amidation to assume a biologically active form in tissues.

AM is produced in endothelial cells and vascular smooth muscle cells.[5] The mRNA of AM has been detected in normal adrenal medulla, heart, kidney, and lung. AM receptors are expressed in both vascular smooth muscle cells and vascular endothelial cells. AM is a vasodilator through its direct action on vascular smooth muscle to increase intracellular cAMP and the stimulation of endothelial nitric oxide release.[6] Cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) stimulate the secretion of AM in vitro, suggesting that AM interacts with the immune system.[7] In addition, we have recently reported that the levels of AM are raised in plasma from patients with systemic sclerosis complicated by pulmonary hypertension.[8]

Immunoreactive AM has recently been found in the cat iris, ciliary body, and aqueous humor; AM also plays an important role in controlling intraocular pressure.[9,10]

Table 1. Clinical features of Behçet's disease patients

Case	Sex/age	Type	Duration	OU	GU	Eye	Skin	Entero	Neuro	Arthritis	Therapy
1	M/53	C	19	+	+	PU,Ir	−	+	−	−	C,P,Cs
2	F/56	IC	17	+	−	PU,Ir	−	−	−	−	N
3	F/51	C	14	+	+	i	EN	−	−	+	AZ
4	M/64	C	21	+	+	Ir	EN,T	−	−	−	C,P
5	M/41	C	10	+	+	PU	EN,T	+	−	+	P,S,M,Cs
6	F/44	C	8	+	+	PU	−	−	−	−	C,P,M
7	M/55	IC	17	+	−	AU	acne	−	−	−	Cs
8	M/61	IC	21	+	−	PU	−	−	+	−	S,M
9[a]	M/33	IC	7	+	−	PU,Ir	−	−	−	−	P,Cs
10	M/43	C	29	+	+	PU	EN	−	−	−	C
11	M/49	C	12	+	+	PU,Ir	T	−	−	−	P,N
12	M/47	IC	15	+	+	i	−	−	−	+	−
13	F/46	C	12	+	+	PU	−	−	−	−	P
14	F/38	IC	10	+	+	PU	−	−	+	+	M
15	F/62	C	40	+	+	AU	−	−	−	−	−
16	F/53	C	6	+	+	AU	EN	−	−	−	C
17	M/64	C	15	+	+	PU	acne	−	−	−	C,M
18	F/36	IC	8	+	+	PU	−	−	−	−	−

OU, oral ulcer; GU, genital ulcer; C, complete type; IC, incomplete type according to the Japanese Behçet's Disease Research Committee Criteria; PU, pan uveitis; Ir, iritis; i, inactive; AU, anterior uveitis; EN, erythema nodosum; T, thrombosis; C, colchicines; P, prednisolone; Cs, ciclosporin; N, nonsteroidal anti-inflammatory drugs; AZ, azathioprine; S, salazosulfapyridine; M, mizoribine

[a] Patient 9 had ocular attack

Because vasculitis, endothelial damage, and ocular involvement are common in BD, AM interacts with the immune system, and the endothelium is a major source, we hypothesized that AM might be involved in the pathogenesis of BD. This study evaluated the association of plasma AM with BD in patients with ocular complications.

Patients and methods

Patients

Eighteen consecutive BD patients with eye complications included 17 patients without ocular attack, in the inactive phase (9 men and 8 women), and 1 male patient with ocular attack, and aged 33 to 64 years (Table 1). Another 6 consecutive BD patients with ocular attack (4 men and 2 women), aged 22–46 years, and 10 normal volunteers (5 men and 5 women), aged 29 to 40 years, were also evaluated (Table 2). All patients were Japanese. The diagnosis of BD was made by Behçet's Disease Research Committee Criteria.[11] Patients with heart failure, hypertension, renal failure, systemic infection and diabetes were excluded. All patients had ocular complications. All systemic neural, enteric, or vascural symptoms were inactive. The diagnosis of uveitis was made by several ophthalmologists according to the International Uveitis Study Group guidelines.[12] We obtained informed consent from all patients and volunteers.

AM analysis

Concentrations of total and mature AM in plasma were measured by immunoradiometric assay (IRMA)[13] in which two different monoclonal antibodies were used. One antibody recognizes the ring structure and the other recognizes the C-terminus of AM.

Table 2. Clinical features of Behçet's disease patients with ocular attack

Case	Sex/age	Type	Duration	OU	GU	Skin	Therapy
1	M/22	IC	6	+	−	+	Cs. C
2	M/41	C	8	+	+	+	Cs. C
3	F/42	C	18	+	+	+	Cs
4	M/36	IC	20	−	−	+	Cs
5	M/43	C	15	+	+	+	Cs. C
6	F/46	C	20	+	+	+	Cs. C

OU, oral ulcerations; GU, genitourinary; IC, incomplete type; C, complete type; Cs, ciclosporin; C, colchicine

ET-1 analysis

Plasma levels of ET-1 in 13 BD patients and 9 normal volunteers were measured by radioimmunoassay (RIA) using antiserum for AM (1–52) NH_2 after the extraction of plasma. We also measured levels of C-reactive protein (CRP) in plasma.

Statistical analysis

Significance was analyzed using the Mann–Whitney *U* test and Speaman's rank correlation test. The results were expressed as means ± SD or box plots and considered significant when the *P* value was <0.05.

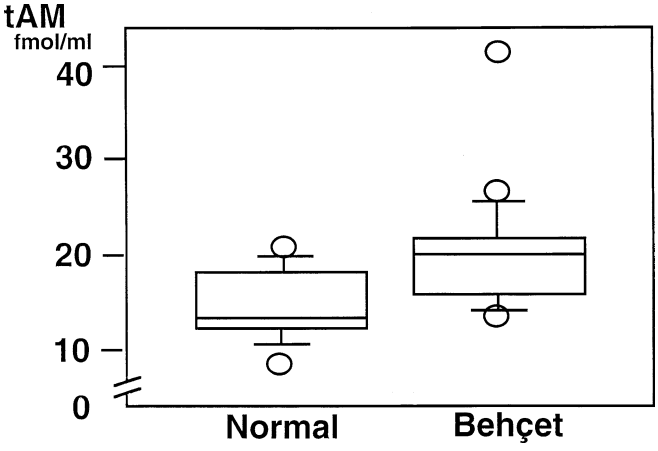

Fig. 1. Concentrations of total adrenomedullin (*tAM*) in the plasma in Behçet's disease (BD; *Behçet*) and *normal* volunteers. Values are represented as box plots; *upper* and *lower bars* show 90th and 10th percentiles, respectively; *upper*, *center*, and *lower lines of the box* show 75th, 50th, and 25th percentiles, respectively

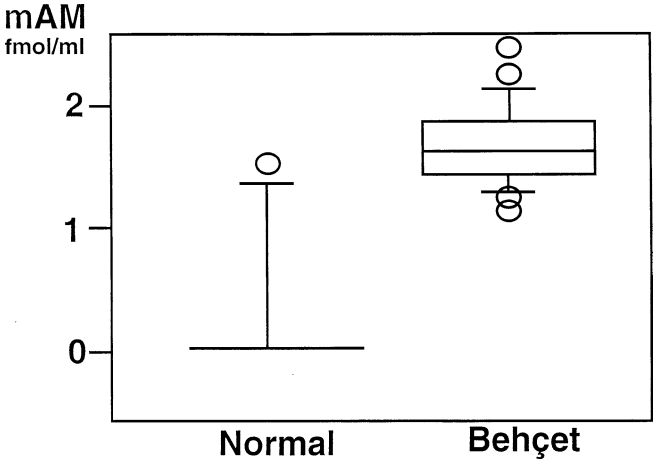

Fig. 2. Concentrations of mature AM (*mAM*) in the plasma in BD and normal volunteers (see legend of Fig. 1)

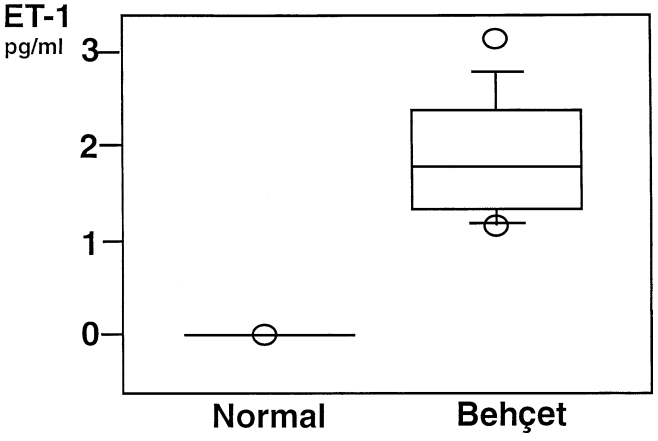

Fig. 3. Concentration of endothelin 1 (*ET-1*) in the plasma in BD and normal volunteers (see legend of Fig. 1)

Results

All patients with BD (n = 18) had recurrent aphthous oral ulcerations. Fourteen patients (77.8%) had recurrent ulcers of the genitalia. Eight patients (44.4%) had cutaneous lesions. All patients had ocular lesions; 12 patients had panuvetitis, 3 patients had anterior uvetitis, 1 of whom had secondary glaucoma due to episodes of attack of anterior uveitis, and 5 patients had iritis. Gastrointestinal symptoms had been present in 2 patients (11%), 1 of whom was diagnosed as entero-BD. Four patients (22%) had arthritis and 2 patients (11%) had shown neurological symptoms.

Concentrations of total AM in the plasma (19.6 ± 6.9 fmol/l) were significantly higher in the 18 consecutive patients with BD than in normal volunteers (14.5 ± 3.6 fmol/l) (P = 0.01) (Fig. 1). The levels of mature AM were also higher in the 18 consecutive patients with BD (1.6 ± 0.4 fmol/l) than in normal volunteers (0.3 ± 0.6 fmol/l) (Fig. 2) (P = 0.0002). The patient among the 18 consecutive patients who had ocular attack had the highest plasma AM level. Thus, we hypothesized that the plasma AM level might be higher in patients with ocular attack than in those without ocular attack.

We measured the AM level in 6 other consecutive patients with ocular attack. The levels of total AM were higher in the 6 patients with ocular attack (19.7 ± 6.4 fmol/l) than in the normal volunteers but did not differ from those in the 17 BD patients in the inactive phase (data not shown). The levels of ET-1 were higher in 13 BD patients (2.0 ± 0.6 fmol/l) than in normal volunteers ($P < 0.0001$) (Fig. 3). The levels of CRP (0.2 ± 0.4 mg/dl) in 13 BD patients were significantly correlated with AM levels (P = 0.01). The levels of ET-1 were not correlated with CRP.

The levels of mature AM were significantly higher in patients with longer disease duration (1.68 ± 0.43 fmol/l) than in patients with shorter disease duration (1.38 ± 0.25 fmol/l) ($P < 0.05$). However, the levels of total AM were not significantly higher in patients with longer disease duration (>15 years) (19.0 ± 5.2 fmol/l) than in patients with shorter disease duration (<15 years) (17.6 ± 3.5 fmol/l) (P = 0.34).

Discussion

Our study demonstrated that the plasma levels of both total and mature AM were elevated in BD patients compared with those in normal controls. Recently, Everekliogl et al. also have reported that AM is elevated in BD patients.[14] Because inflammatory cytokines such as TNF-α or IL-1β stimulate AM secretion from endothelial cells,[7] levels of circulating TNF-α and IL-1β may be elevated. These cytokines may be involved in the activation of neutrophils and augmented cellular interactions between neutrophils and endothelial cells as a result of enhanced expression of adhesion molecules.[7] Vascular injuries are superimposed on the hypercoagulability that is also characteristic of BD and

which may be due in part to activated endothelial cells and activated platelets. Thus, the elevation of plasma levels of AM found in our patients may be a consequence of endothelial damage.

Because all BD patients in this study were outpatients at the ophthalmic clinic, all patients had eye complications. Thus, we studied two groups of BD patients, one group of BD patients who had active eye complications and the other group of BD patients whose eye complications were inactive. We found that the levels of AM did not differ between patients with ocular attack and patients in the inactive phase and between anterior uveitis and panuveitis. It is speculated that ocular attack may not elevate the plasma AM level because the volume of ocular organs is small.

Structurally, AM belongs to the calcitonin gene-related peptide (CGRP) superfamily and elicits a potent vasodilator effect. CGRP can play a role in inflammatory responses of the eye, which is supported, moreover, by the presence of CGRP receptors in the iris and ciliary body.[15] Recently, immunoreactive AM has been found in the cat iris, ciliary body, and aqueous humor. Clementi et al. reported that AM causes a dose-dependent conjunctival hyperemia, accompanied by an increase in inflammatory cell number and prostaglandin E_2 concentration in the aqueous humor and by an increase of uveal vascular response and myeloperoxidase activity.[9] They also reported that this effect involves the nitric oxide system acting through specific AM receptors.[9] In addition, AM-induced ocular inflammation and vascular compromise is a particularly prominent feature, evidenced by the number of inflammatory cells found in the aqueous humor and extravascular uveal tissue.[9] Thus, we speculate that AM may play an important role in ocular complications in patients with BD, although it remains to be shown whether AM levels are higher in BD patients with ocular complications than in BD patients without ocular complications. We are now measuring AM levels in BD patients without ocular complications.

AM has been reported as a mediator of inflammation to stimulate the production of IL-6 by fibroblasts.[16] Because levels of total AM were correlated with CRP levels in BD patients in the inactive phase, AM may be a useful marker for monitoring disease activity. However, AM exerts anti-inflammatory effects by inhibiting the production of a chemoattractant from alveolar macrophages.[17] Moreover, we recently demonstrated that AM inhibits IL-6 production in rheumatoid arthritis synoviocytes.[18] Thus, AM may also play a role as an antiinflammation factor in BD patients.

In summary, we demonstrated that both total and mature AM levels were elevated in plasma from patients with BD. AM may play an important role as an antiinflammation factor or may reflect endothelial damage as a marker of disease activity in BD patients. Further studies are needed to clarify the roles of AM in the pathogenesis of BD.

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