Blocking TGF-β type 1 receptor partially reversed skin tissue damage in experimentally induced atopic dermatitis in mice

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Keywords:
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Interleukin (IL)-1β/6
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Transforming growth factor (TGF)–β1
Transforming growth factor–β type 1 receptor (TGF–βR1)
Tumor necrosis factor (TNF)–α

A B S T R A C T
Animals with impaired transforming growth factor (TGF)–β1 signaling developed spontaneous lethal autoimmune inflammation and autoimmune diseases. Moreover, evidence for modified TGF–β signaling in atopic dermatitis (AD) exists. Therefore, the goal of this study was to determine whether SB-431542, a potent and selective inhibitor of the TGF–β type 1 receptor (TGF–βR1), could attenuate such a severe reaction in mice. In addition, the molecular underpinnings the possible protective effects were also investigated. Repeated epicutaneous application of DNCB was performed on the ear and shaved dorsal skin of mice to induce AD-like symptoms and skin lesions. SB-431542 (1 mg/kg) was given by intra-peritoneal injection three times weekly for 3 weeks to assess the anti-pruritic effects. Serum levels of TGF–β1, TGF–βR1, latency-associated peptide (LAP), tumor necrosis factor (TNF)–α, interleukin (IL)–1β and IL-6 were assessed by ELISA. Moreover, the gene expression of TNF–α, IL–1β and IL-6 were determined. Apoptotic pathway was evaluated by measuring the activity of caspase-3 and by staining skin sections with anti-caspase-3 antibodies. We found that SB-431542 alleviated DNCB-induced AD-like symptoms as quantified by skin lesion, dermatitis score, ear thickness and scratching behavior. In parallel, SB-431542 blocked DNCB-induced elevation in serum levels of TNF–α, TGF–β1, TGF–βR1, LAP, IL–1β, IL-6 and IgE. The collective results indicate that SB-431542 partially suppresses DNCB-induced AD in mice via reduction of TGF–β1 signaling pathway associated with inhibition of inflammation and apoptosis.

1. Introduction
Atopic dermatitis (AD), also known as atopic eczema, is a chronic relapsing inflammatory disease of the skin usually develops in early childhood. Atopy is defined as an inherited tendency to produce immunoglobulin E (IgE) antibodies in response to minute amounts of common environmental proteins [1]. It is clinically distinguished by pruritus, eczematous plaques and a defective epidermal barrier [2]. Dermatitis and eczema are often used synonymously, although the term eczema is sometimes reserved for the acute manifestation of the disease. It affects 10–20% of children and 3% of adults. Global evidences reflect a marked increase in prevalence, which has tripled since 1960 [3]. Data derived from the international study of asthma and allergies in childhood (ISAAC) has shown a worldwide prevalence ranging from 2% to 20% with a tendency for higher prevalence in affluent European and Australasian settings, and rising eczema burden in most developing country settings and in younger children [4].

Most cases of childhood AD are mild with infrequent exacerbations and minimal impact to quality of life. However, more severe AD is correlated with poorer overall health, impaired sleep and increased healthcare utilization [5]. There also appears to be an association between severe AD and multiple comorbid chronic health disorders, both atopic and nonatopic, such as asthma, hay fever, food allergies, recurrent ear infections, visual problems and impaired dental hygiene [6]. It is widely accepted now that AD is a systemic disease with atopic and nonatopic comorbidities [7,8]. AD therapeutic strategies depends mainly on application of local or systemic corticosteroids, which have a high level of collateral and undesirable effects [9]. Therefore, the search for new active agents against dermatitis is of great interest.

Transforming growth factor (TGF)–β1 is a multifunctional cytokine and its signaling is essential for the maintenance of immune tolerance [10]. Briefly, TGF–β1 signaling is initiated by binding of TGF–β1 to its heterodimeric transmembrane receptor complex, which is composed of type I (TGF–βR1) and type II (TGF–βRII) receptors. Binding of TGF–β1 results in the phosphorylation and activation of TGF–βRI, which then activates TGF–βRII [11,12]. Animals with impaired TGF–β signaling developed spontaneously lethal autoimmune inflammation and diseases [13,14]. Moreover, evidence for modified TGF–β signaling in AD exists [15,16]. Furthermore, TGF–β1 signaling is important for the local immune response in AD [17]. Therefore, the goal of this study was to...
determine whether SB-431542, a potent and selective inhibitor of the TGF-β1 receptor, could attenuate such a severe reaction in mice. In addition, the molecular underpinnings of the possible protective effects were also investigated.

2. Materials & methods

2.1. Animals and their treatment outlines

The animal protocol was approved by the local ethical committee. Four-week-old BALB/c mice were housed under specific pathogen-free conditions at 22 ± 2 °C with a 12-h light-dark cycle. Mice were classified into the following groups with 10 mice each:

1. **Control group.** Mice received intra-peritoneal (ip) CMC three times weekly for three weeks.
2. **Control shaved group.** The dorsal hair of mice was removed and a solution of acetone : olive oil, 3:1 was applied on the back skin, face, and back of both ears.
3. **SB-431542-treated control group.** Mice were injected with 1 mg/kg SB-431542 ip (Selleck Chemicals, Houston, TX, USA) three times weekly for three weeks.
4. **DNCB-induced AD group.** AD-like skin lesions were induced in mice using 2,4-dinitrochlorobenzene (DNCB). The dorsal hair of mice was removed. After 24 h, 100 µl of 1% DNCB solution (acetone : olive oil, 3:1) was applied on the back skin and 10 µl each were applied to the face and the back of both ears (day –4) for sensitization. Five days after dorsal hair removal, 0.2% DNCB was applied to challenge the dorsal skin (150 µl), the face, and the back of both ears (10 µl each) three times a week for 3 weeks [18,19].
5. **SB-431542 treated group.** Mice were treated with DNCB for 3 weeks followed by 1 mg/kg of SB-431542 ip three times/week for another three weeks.

2.2. Measurement of scratching behavior frequency

Mice were placed into cages and the number of scratching behaviors was counted for 10 min. Measurement was repeated for five times in the last two days. The scratching behavior was defined as the movement with hind paws in this experiment.

2.3. Skin lesion, dermatitis score and ear thickness

To compare the improvement of skin condition by treatment with SB-431542, mice were anesthetized and pictures were taken for mice on the last day before sacrifice. The dermatitis score was measured once a week. Scores of 0 (none), 1 (mild), 2 (moderate), and 3 (severe) were given for each of the four symptoms: (i) erythema/hemorrhage, (ii) edema, (iii) excoriation/erosion, and (iv) scaling/dryness. A total dermatitis score indicating clinical severity was defined as the sum of all scores (maximum score: 15). Mouse ear thickness was also measured and recorded once a week using a caliper.

### Table 1

<table>
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<th>Primer Accession number</th>
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<tr>
<td></td>
<td>5′- CAGCTTGTCCCCCTGAGAAACC -3′</td>
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<tr>
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<td>GAPDH M32599</td>
<td>5′- ACCACAGCTCAGGATCCATCAC -3′</td>
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Fig. 1. Effect of atopic dermatitis (AD) alone and in combination with 1 mg/kg SB-431542 on serum levels of transforming growth factor-β type 1 receptor (TGF-βR1, a), transforming growth factor (TGF)-β1 (b), latency-associated peptide (LAP, c). †: significant difference compared with the control groups at p < 0.05. $: significant difference compared with atopic dermatitis group at p < 0.05.
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