A new selective vascular endothelial growth factor receptor 2 inhibitor ablates disease in a mouse model of psoriasis

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Abstract. Psoriasis is a common chronic inflammatory skin disease and its underlying pathogenesis is still not fully understood. Therapeutic interventions are currently limited and restricted to the treatment of symptoms rather than targeting the mechanisms underlying the disease. Vascular remodeling is a hallmark of psoriasis; however, anti-vascular strategies to treat psoriasis have received little attention to date, particularly systemic treatment with a small molecule compound. The aim of this study was to investigate the anti-inflammatory effect of a newly identified vascular endothelial growth factor (VEGF) receptor 2 inhibitor, SKLB1002, and its possible mechanism of action in a transgenic mouse model of psoriasis. Fifteen 8-12-week-old K14-VEGF transgenic mice received consecutive intraperitoneal (i.p.) injections of SKLB1002, vehicle or saline for 4 weeks. After 4 weeks of treatment, the disease symptoms were assessed and histological analyses were performed on ear sections by hematoxylin and eosin (HE) and immunohistochemistry staining. Systemic treatment with SKLB1002 reduced symptoms of ear inflammation in K14/VEGF transgenic mice, the pathological score was significantly decreased, and acanthosis, focal parakeratosis, hyperkeratosis and hemangiectasis were improved. Furthermore, systemic treatment with SKLB1002 significantly reduced vascular abnormalities, permeability and T-cell infiltration. These results demonstrated that targeted inhibition of VEGFR2 by a small molecule inhibitor is an effective method, which may be a new therapeutic option for psoriasis therapy.

Introduction

Psoriasis is a chronic inflammatory skin disease that is characterized by erythema, epidermal hyperplasia, inflammatory infiltrates, and enlarged, tortuous and hyperpermeable blood vessels (1,2). Previous studies have shown that the endothelial microvascular bed was increased four-fold in psoriatic skin compared with normal skin (3). Dermal microvascular expansion with abnormal orientation, and dilatation of capillaries in the biopsies of the psoriatic skin revealed that the disease was angiogenesis dependent (4,5) and signified the importance of angiogenesis in psoriasis.

Numerous growth factors and cytokines are involved in angiogenesis. Of all known angiogenic molecules, vascular endothelial growth factor (VEGF) is the key mediator of angiogenesis. Several studies indicate a vital role of VEGF in the pathogenesis of psoriasis: epidermal-derived VEGF is highly upregulated in psoriatic skin lesions (6-8); VEGF serum levels are correlated with disease severity (9); VEGF expression in basal keratinocytes showed psoriatic-like skin inflammation with increased tortuosity and branching of dermal blood vessels in a mouse model (10,11). VEGF receptors 1 and 2 were expressed on the surface of keratinocytes, and the VEGF secreted by the keratinocytes was able to bind to these receptors and activate the signaling pathway in an autocrine manner (12-14). Thus, anti-vascular strategies to treat psoriasis by blocking VEGF binding to VEGFR are anticipated, although to date the majority of anti-angiogenic approaches have primarily focused on the development of cancer therapeutics (15-17).

In the present study, we used a therapeutic approach in a transgenic mouse model (18) of chronic, psoriasis-like skin inflammation, using the anti-VEGFR2 small molecule compound SKLB1002. Systemic treatment of transgenic K14-VEGF mice with SKLB1002 strongly reduced skin inflammation in contrast with control animals. The mice showed a marked improvement in psoriatic phenotype, normalization of the epidermal architecture, and a decrease in the number and size of blood vessels. Furthermore, the immune infiltrate in the skin was reduced in SKLB1002-treated mice.

Materials and methods

Animals. K14-VEGF transgenic homozygous mice (18) displaying symptoms of human psoriasis were provided by the State Key Laboratory of Biotherapy and Cancer Center, Sichuan University (Sichuan, China). Eight to twelve-week-old mice with moderate to serious psoriasis were selected for the experiment. The mice were housed under specific pathogen-free (SPF) conditions. Animal experiments were approved by the Institutional Animal Care and Use Committee of Southwest University for Nationalities (Chengdu, Sichuan, China).
SKLB1002. The small molecule compound was donated by Dr Sheng-Yong Yang from the State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University (Sichuan, China).

Antibodies. For immunohistochemistry, rabbit anti-mouse antibodies against K6 (Abcam, Cambridge, MA, USA), CD4 (Abcam), CD8 (Abcam), CD31 (Abcam), VEGF (Boster, Fremont, CA, USA), CD54 (Boster) and E-selectin (Boster) were used.

In vivo treatment with the tyrosine kinase inhibitor SKLB1002. K14-VEGF transgenic homozygous mice were divided into three groups as follows: a treatment group receiving SKLB1002 and control groups receiving only vehicle or saline. Mice were dosed with SKLB1002, vehicle or saline once daily by intraperitoneal (i.p.) injection for four weeks. Mice in the treatment group received 50 mg/kg SKLB1002 (dissolved in 5% DMSO + 35% PEG400 suspension, i.p.), which was a dose previously determined to be effective in preparatory experiments (data not shown). Animals in two control groups were treated with the same volume of vehicle (5% DMSO + 35% PEG400) and saline, respectively. Twenty-four hours after the final treatment, photographs of mouse ears were obtained before all mice were sacrificed. Ear samples were fixed in 4% neutral buffered paraformaldehyde for histological analysis.

Histology. Paraffin-embedded skin sections (~5 µm) from treated-K14/VEGF mice were stained by HE and immunohistochemistry. Quantitative assessments of the pathological score, based on Baker's method (19), were performed in five randomly selected high-power fields (x400) in each HE-stained section to assess the severity of psoriasis. The mean epidermal thickness of the ear skin was also measured as an indication of epidermal proliferation. Immunohistochemistry staining was performed according to the antibody protocol, and images were acquired using an Olympus BX60 microscope (Olympus, Japan). Paraffin sections were deparaffinized and rehydrated, then heat-induced antigen retrieval was required for 5 min prior to immunohistochemical staining. The tissue sections were incubated with the primary antibodies overnight at 4°C. Cells were counted in five randomly chosen fields (x400) in each immunohistochemically stained section (n=5 for each group). The evaluations were performed by two blinded observers.

Statistical analysis. SPSS 16.0 was used for statistical analysis (SPSS, Inc., Chicago, IL, USA). Data are expressed as the means ± SD. The statistical analysis in all experiments was performed using one-way analysis of variance (ANOVA) or t-test. P<0.05 was considered to indicate a statistically significant result.

Results

Systemic treatment with SKLB1002 reduces symptoms of ear inflammation in K14/VEGF transgenic mice. The effects of a newly identified VEGF receptor tyrosine-kinase inhibitor, SKLB1002, were tested in K14/VEGF transgenic mice exhibiting numerous characteristic features of psoriasis. Fifteen 8-12-week-old K14-VEGF transgenic mice received consecutive i.p. injections of SKLB1002, vehicle or saline for 4 weeks. The mice treated with the vehicle or saline exhibited focal skin lesions on their ears, which were highly similar to human psoriasis. By comparison, the mice treated with SKLB1002 showed only hyperaemia and very slight increscence of the skin on their ears (Fig. 1). SKLB1002 treatment was well-tolerated; the mice showed no signs of sickness and did not lose weight (data not shown).

SKLB1002 treatment normalizes the epidermal architecture in inflamed skin. To better characterize the efficacy of systemic VEGF blockade in reducing psoriasis-like skin inflammation, histological analyses were performed on ear sections obtained from K14/VEGF littermates treated with SKLB1002, vehicle and saline. After 4 weeks of treatment, HE-stained sections revealed the typical histopathological signs of the psoriasis-like phenotype in the control-treated mice, including acanthosis (thickened epidermis), epidermal rete elongation, focal parakeratosis (retention of nuclei in the stratum corneum), hyperkeratosis (thickening of the stratum corneum), hemangiectasia, abundant infiltrated lymphocytes and microabscesses. By contrast, systemic inhibition of VEGF led to a notable reduction in psoriasis-like histological features (Fig. 2).

As shown in Fig. 2, SKLB1002 treatment led to a significant improvement in skin inflammation, which was confirmed by the pathological score for HE-stained preparations based on the Baker score system. The pathological scores of the mice treated with SKLB1002 were significantly different (n=5 in each group; P<0.01, one-way ANOVA) from controls. In addition, the average epidermal thickness was reduced by 80% in the group treated with SKLB1002 when compared with the control groups (n=5 in each group; P<0.01, one-way ANOVA), whereas the difference between the vehicle-treated group and saline-treated group was not significant. During psoriatic hyperproliferation of keratinocytes, the epidermal hyperproliferation marker keratin 6 displayed a much broader staining pattern. Treatment with SKLB1002 significantly inhibited the expression of keratin 6 in the epidermis. By contrast, keratin 6 expression was marked in the epidermis of vehicle-treated mice. Thus, inhibition of VEGF normalized the epidermal skin architecture in this mouse model of psoriasis.

Decrease in T-cell infiltration mediated by SKLB1002 in vivo. The defining histological features of psoriasis include marked infiltration of T cells. We thus performed immunohistochemical assays to examine the variations in CD4+ and CD8+ cells in skin sections. As shown in Fig. 3, when compared with the control, SKLB1002 treatment significantly decreased the number of CD4+ and CD8+ cells by 71.2% and 78.5%, respectively, which demonstrated inhibition of the infiltration of inflammatory T lymphocytes.

SKLB1002 significantly reduces vascular abnormalities and permeability in vivo. As shown in Fig. 4A, when compared with the control, SKLB1002 treatment significantly decreased the expression of VEGF and CD31, which indicated the suppression of the proliferation and dilation of blood vessels. Notably, the average number of blood vessels was significantly
reduced (P<0.01) and the average size of blood vessels (P<0.01) was significantly smaller in SKLB1002-treated mice than in vehicle control-treated animals.

In particular, the expression of specific endothelial cell adhesion molecules is a hallmark of the hyperplastic and inflamed vessels observed in human psoriatic skin lesions, including the expression of E-selectin, VCAM-1 (CD106) and ICAM-1 (CD54). Compared with the vehicle treatment group, SKLB1002 treatment significantly inhibited the expression of E-selectin, CD54 and CD106 (Fig. 4B). Thus, inhibition of
Overexpression of VEGF model. The three B-phenotypes completely cure psoriasis in a mouse model. Mechanistic studies described SKLB1002 effectively alleviates skin inflammation or infiltration observed in the skin. Most likely contributed to the diminished inflammatory cell infiltration observed in the skin. T lymphocytes by systemic treatment with VEGFR inhibitor SKLB1002 in K14/VEGF transgenic mice. Besides its role in angiogenesis, VEGF induces hyperpermeability of blood vessels, leading to tissue edema during inflammation (22-24). Furthermore, chronic overexpression of VEGF in the skin of K14-VEGF transgenic mice promoted leukocyte rolling and adhesion in skin microvessels, most likely resulting from the increased expression of adhesion molecules such as E-selectin, CD106 and CD54 (18,25). Thus, the inhibition of these additional activities of VEGF related to attracting and activating T lymphocytes by systemic treatment with VEGFR inhibitor most likely contributed to the diminished inflammatory cell infiltration observed in the skin.

In conclusion, the new and potent VEGFR2 inhibitor SKLB1002 effectively alleviates skin inflammation or completely cures psoriasis in a mouse model. Mechanistic studies indicated that SKLB1002 treatment significantly decreased the number of blood vessels and reduced the tortuosity of epidermal blood, prevented vascular leakage and T lymphocyte infiltration in skin, which may promote skin inflammation. These findings establish a solid basis for future clinical studies of the new selective VEGFR2 inhibitor for the treatment of psoriasis.

Discussion
Psoriasis is a common inflammatory disease and its underlying pathogenesis is still not fully understood (20,21). Therefore, therapeutic interventions are currently limited and restricted to the treatment of symptoms rather than targeting the mechanisms underlying the disease. In the present study, we investigated the activity of a new small molecule VEGF inhibitor in a psoriasis-like mouse model. The three major components of psoriasis pathogenesis, infiltration of leukocytes, hyperproliferation of epidermal keratinocytes, and occurrence of vascular abnormalities, were markedly improved following treatment with SKLB1002. These findings indicate that therapeutic intervention at the level of the vasculature may be sufficient to reduce the immune-mediated and epidermal components of the disease.

SKLB1002 is a new and selective VEGFR2 inhibitor that potently inhibits VEGFR2 with a half maximal inhibitory concentration (IC_{50}) of 32 nmol/l (16). SKLB1002 significantly inhibits HUVEC proliferation, migration, invasion and tube formation in vitro (16). In this study, vascular abnormalities and secretion of VEGF were reduced by treatment with SKLB1002 in K14/VEGF transgenic mice. Besides its role in angiogenesis, VEGF induces hyperpermeability of blood vessels, leading to tissue edema during inflammation (22-24). Furthermore, chronic overexpression of VEGF in the skin of K14-VEGF transgenic mice promoted leukocyte rolling and adhesion in skin microvessels, most likely resulting from the increased expression of adhesion molecules such as E-selectin, CD106 and CD54 (18,25). Thus, the inhibition of these additional activities of VEGF related to attracting and activating T lymphocytes by systemic treatment with VEGFR inhibitor most likely contributed to the diminished inflammatory cell infiltration observed in the skin.

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References


