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Baicalein protects normal human epidermal keratinocytes against bullous pemphigoid immunoglobulin G-induced alteration

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ABSTRACT

Background: Bullous pemphigoid (BP) is a common autoimmune bullous dermatosis in the elderly, with immunoglobulin G (IgG) autoantibodies playing a major role in its pathogenesis. Kampo medicine has been used to ameliorate BP.

Objective: To determine whether baicalein, a major compound in Ou-gon, a crude drug used in Kampo medicine, protects normal human epidermal keratinocytes (NHEKs) against BP-IgG-induced damage.

Methods: Microscopic examination of living cells, ELISA, a SYTOX green uptake assay, and an intracellular reactive oxygen species (ROS) accumulation assay were used to determine the effects of baicalein on BP-IgG-induced cell damage.

Results: We found that 2 mg·mL⁻¹ BP-IgG induced cell detachment, interleukin (IL)-6 and IL-8 production, and intracellular ROS accumulation in NHEKs. In contrast, these BP-IgG-induced reactions were significantly prevented by 100 μg·mL⁻¹ baicalein in NHEKs ($p < 0.05$).

Conclusions: This study provides the evidences that traditional Kampo medicine containing baicalein is efficacious against BP.

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In the skin, hemidesmosome connects basal keratinocytes to the basement membrane, and two major hemidesmosome proteins, BPAG2/collagen XVII (BP180) and BPAG1/dystonin (BP230), play a pivotal role in adhering basal keratinocytes to the basement membrane [1–5]. BP230 is associated with intracellular hemidesmosomal plaques and BP180 links basal keratinocytes to the basement membrane [1]. Bullous pemphigoid (BP) is a common autoimmune bullous dermatosis in elderly population, characterized by large and tense blisters. Immunoglobulin G (IgG) autoantibodies against BP180 play an important role in BP pathogenesis [1–5]. Some studies have reported that BP IgG binding to BP180 alone cannot induce blister formation, and BP IgG binding to BP180 followed by complement activation, granulocyte infiltration, and its activation are required for dermal–epidermal separation in BP [1]. Recent studies have revealed that BP IgG can induce blister formation in a complement-independent manner [2–4], and a complex of BP180 and IgG autoantibodies is internalized, disrupting adhesion between keratinocytes and the basement membrane [2,5]. The Kampo medicines Sho-saiko-to and Sairei-to have been used to relieve bullae formation in patients with BP (Supplemental Table 1). Ou-gon is derived from *Scutellaria*

baicalensis Georgi roots and commonly included in both Sho-saiko-to and Sairei-to. The flavone, 5,6,7-trihydroxyflavone (baicalein) (Supplemental Fig. 1), a major compound in Ou-gon, is known to have anti-inflammatory activity and inhibitory effect on reactive oxygen species (ROS) production in NG108-15 cells [6]. The aim of the present study was to elucidate the action mechanisms of baicalein by investigating its inhibitory effects against anti-BP antibody-induced cell damage, intracellular ROS accumulation, interleukin (IL)-6 and IL-8 production, and cell detachment in cultured normal human epidermal keratinocytes (NHEKs) in vitro.

In the present study, we demonstrated that baicalein protected NHEKs against BP IgG-induced cell damages (Fig. 1 a) and inhibited BP IgG-induced ROS accumulation and IL-6 and IL-8 production by NHEKs in a concentration-dependent manner (Fig. 1b, c). In addition, baicalein inhibited BP IgG-induced cell detachment of NHEKs in tissue culture dishes (Fig. 2a, b). These findings are consistent with those of a previous study, which reported that flavones inhibit ROS production in SH-SY5Y cells [7]. Our data suggest that baicalein prevents BP blister formation by suppressing BP IgG-induced cell damages, decreasing adhering ability, activating superoxide production, and releasing inflammatory cytokines from keratinocytes. Baicalein has also been indicated for other skin diseases such as atopic dermatitis, acute ultraviolet B irradiation-induced skin damage, and skin hypersensitivity [8]. Recently, we have clarified the potent antifungal activity of baicalein [9]. As

Abbreviations: BP, bullous pemphigoid; IgG, Immunoglobulin G; IL, interleukin; NHEK, normal human epidermal keratinocyte; ROS, reactive oxygen species.

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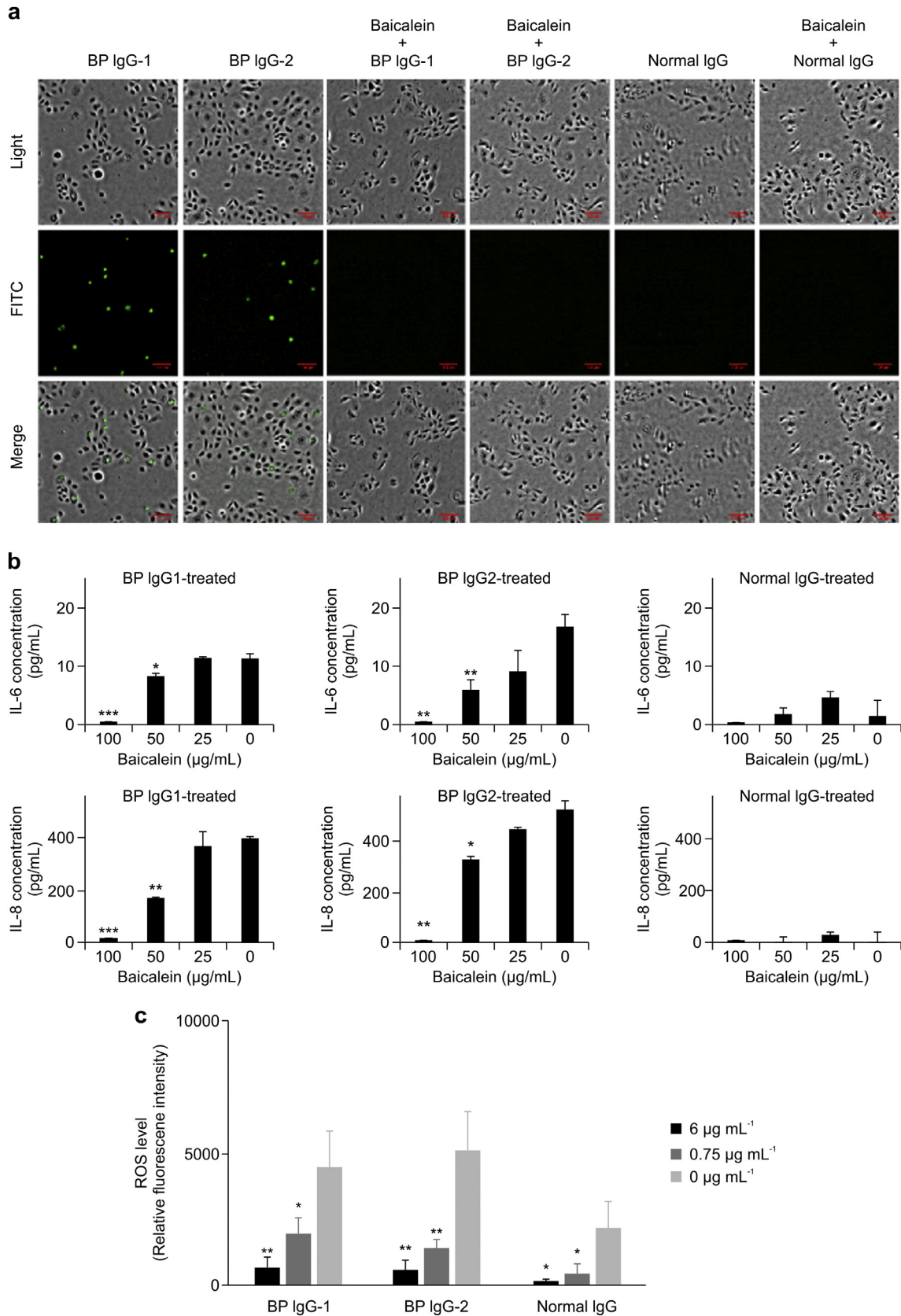


Fig. 1. (a) Cell damage assessed using the SYTOX green uptake assay. NHEKs treated with BP IgG (treatment time: 16 h) were positively stained with SYTOX green, whereas those treated with normal IgG (treatment time: 16 h) were unstained. Baicalein treatment protected NHEKs against BP IgG-induced damage (treatment time: 16 h). The final concentration of BP IgG or normal IgG was 2 mg mL⁻¹ and that of baicalein was 100 µg mL⁻¹. Scale bar, 20 µm. (b) Effects of baicalein on IL-6 and IL-8 production in BP IgG-treated NHEKs. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 compared with 0 µg mL⁻¹ baicalein treatment (treatment time: 16 h). (c) Effects of baicalein on intracellular ROS accumulation in BP IgG-treated NHEKs. Data are presented as mean ± SD of three independent experiments. **p* < 0.05 and ***p* < 0.01, compared with baicalein-free (0 µg mL⁻¹) treatments (treatment time: 6 h). All data are expressed as mean ± standard deviation of three independent experiments.

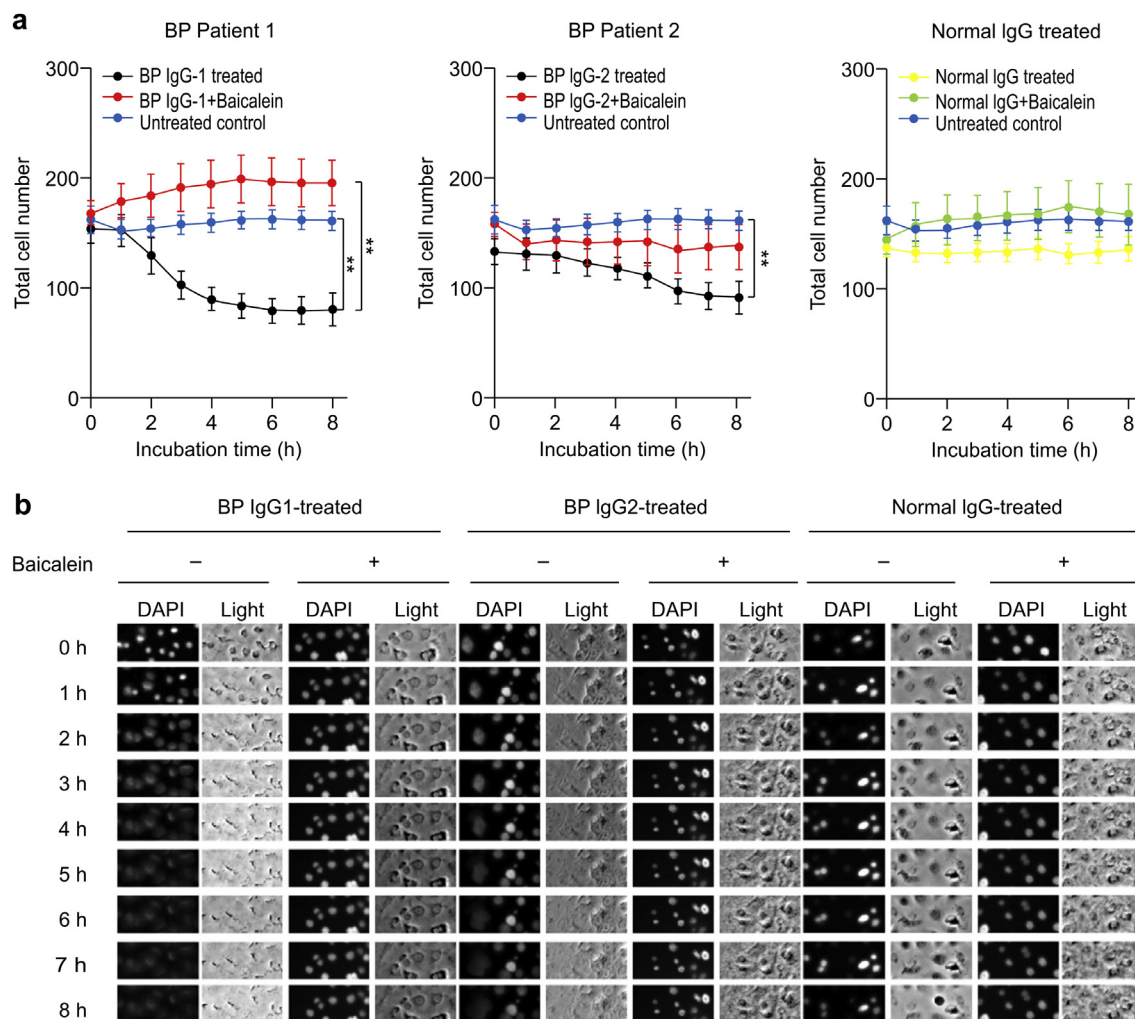


Fig. 2. (a) Effects of baicalein on BP IgG-induced cell detachment. NHEKs were treated with 2 mg mL^{-1} BP IgG in the presence or absence of $100 \text{ }\mu\text{g mL}^{-1}$ baicalein (treatment time: 8 h). Normal IgG-treated (treatment time: 8 h) and untreated NHEKs were used as the controls. Data are presented as mean \pm standard error of three independent experiments; $**p < 0.01$ and $***p < 0.001$. (b) Effects of baicalein on live cell microscopic evaluation. Live cell images were obtained in autofocus at a magnification of $20\times$. NHEKs were incubated with 2 mg mL^{-1} BP IgG (treatment time: 8 h). Normal IgG-treated NHEKs in the presence or absence of $100 \text{ }\mu\text{g mL}^{-1}$ baicalein (treatment time: 8 h). DAPI: 4',6-diamidino-2-phenylindole staining images. Light: bright-field images.

baicalein is only slightly toxic or entirely nontoxic to healthy human cells [10], we propose that baicalein could serve as a new, safe, and effective treatment for BP associated with fungal infections. To understand the action mechanism of baicalein, its in vivo effects against BP should be evaluated.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jdermsci.2019.06.010>.

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