Anti-inflammatory effects of Saxifraga Sarmentosa Extract via regulation of Toll-like receptor 2-mediated innate immune response in epidermal keratinocytes

EP_374

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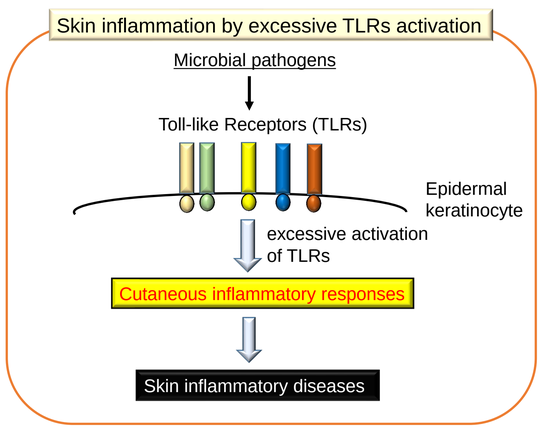
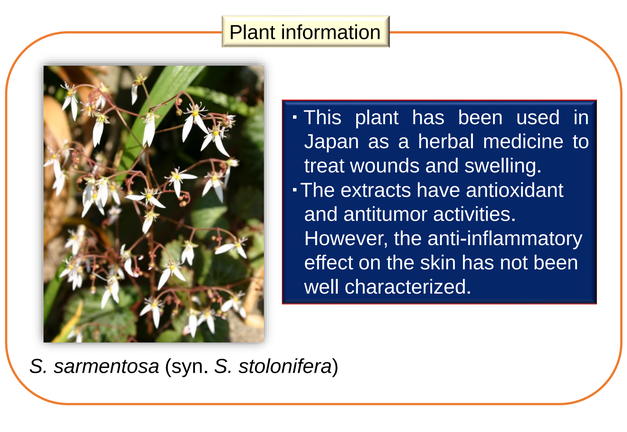
Introduction:

Skin is an organ that is exposed to a myriad of diverse microorganisms, and that serves as a first line of defense, constituting a physical, chemical, and immunological barrier [1]. The innate immune system provides protection against microbial pathogens and plays a vital role in maintaining skin homeostasis [2]. Toll-like receptors (TLRs) are a class of proteins that play a key role in the system. Recognition of microbial components by TLRs initiates signal transduction pathways, which triggers expression of genes involved in skin inflammatory responses [3]. For example, *Cutibacterium acnes* induces inflammatory cytokine production through a TLR2-dependent pathway in acne [4]. In the present study, we aimed to find an active ingredient from medical plants for suppressing excessive inflammatory innate response in epidermal keratinocytes. Consequently, the inhibitory effect of an extract obtained from *Saxifraga sarmentosa* (syn. *Saxifraga stolonifera*, shown below) on TLR2-mediated inflammation was investigated.





Materials & Methods:

＜Materials＞

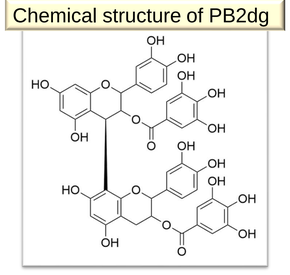
Preparation of plant extracts

Whole plants of *S. sarmentosa* were extracted with 50% EtOH under reflux, and the filtrate was concentrated with an evaporator to obtain the extract, Saxifraga Sarmentosa Extract (SSE).

Fractionation and isolation of SSE components

SSE was subjected to Diaion HP-20 yielding five fractions (H_2O eluate: F1, 30% MeOH eluate: F2, 50% MeOH eluate: F3, 80% MeOH eluate: F4, MeOH eluate: F5). F3 was further fractionated by ODS column yielding five fractions (H_2O eluate: F3-1, 30% MeOH eluate: F3-2, 50% MeOH eluate: F3-3, 80% MeOH eluate: F3-4, MeOH eluate: F3-5). F3-1 was further applied to silica gel column. Finally, the 100% MeOH eluate (F3-1-5) was purified by HPLC using C30 column to isolate components. One of isolated components, procyanidin B2 3,3'-di-*O*-gallate (PB2dg), was shown below.

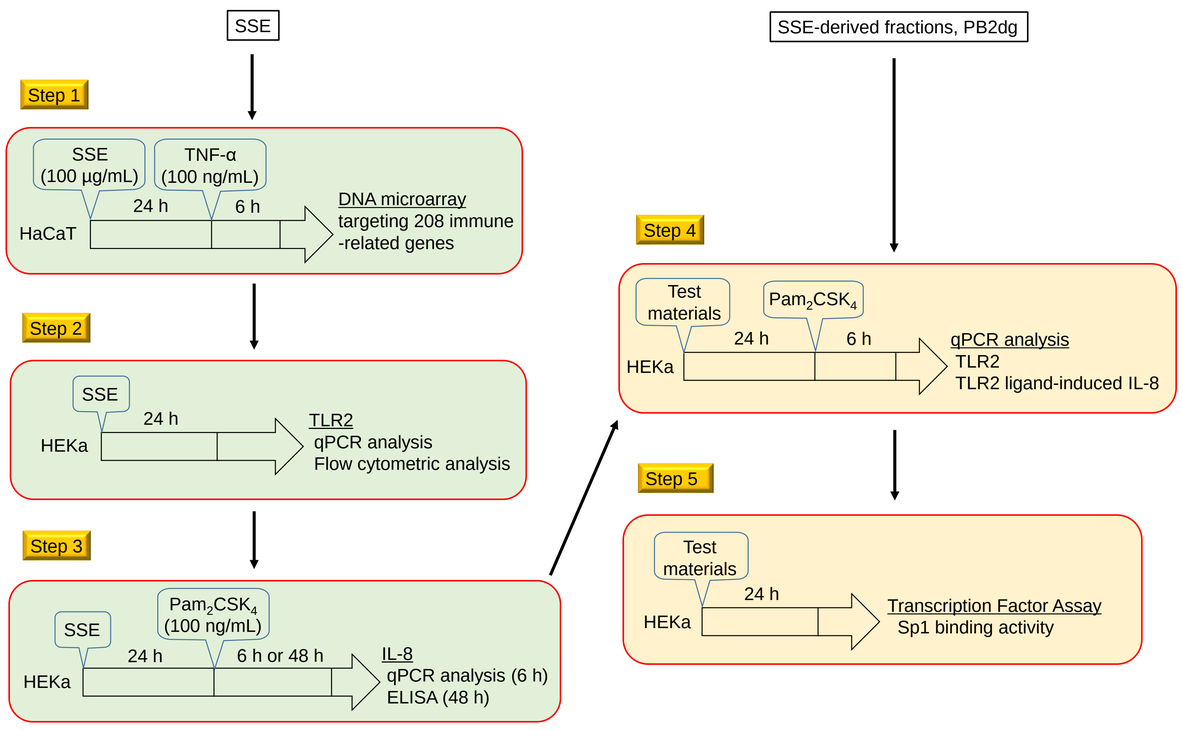
Chemical structure of PB2dg



Cells and cell culture

The human keratinocyte cell line (HaCaT) and adult human epidermal keratinocytes (HEKa) were used for this study.
HaCaT cells and HEKa were cultured in high glucose DMEM supplemented with 10% fetal bovine serum and in EpiLife medium supplemented with EpiLife Defined Growth Supplement, respectively.

＜Methods＞



Results & Discussion:

Step 1

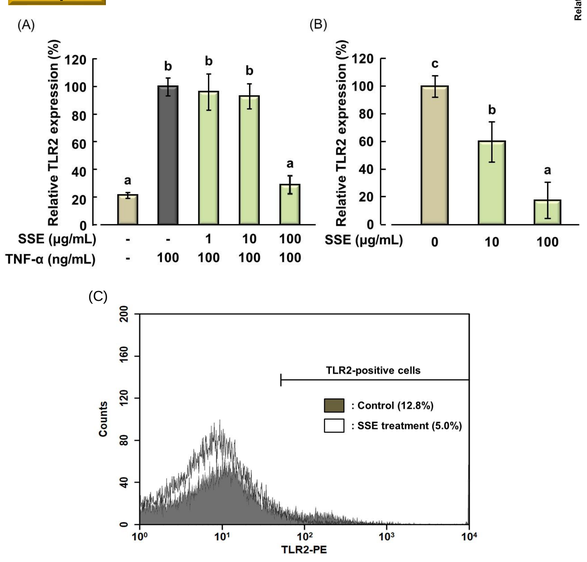
DNA microarray analysis

Gene symbol	Accession number	Gene name/description	SIR
TLR2	NM_003264.3	toll-like receptor 2	-15.66
TLR3	NM_003265.2	toll-like receptor 3	-10.41
IFI16	NM_005531.2	interferon, gamma-inducible protein 16	-4.78
TRIM22	NM_006074.3	tripartite motif-containing 22	-4.69
IFIH1	NM_022168.2	interferon induced with helicase C domain 1	-4.18
IFIT3	NM_001549.3	interferon-induced protein with tetratricopeptide repeats 3, transcript variant 1	-4.13
NMI	NM_004688.1	N-myc (and STAT) interactor	-3.63
TRAF5	NM_004619.3	TNF receptor-associated factor 5, transcript variant 1	-3.41
TANK	NM_004180.2	TRAF family member-associated NFKB activator, transcript variant 1	-3.21
CASP1	NM_033292.2	caspase 1, apoptosis-related cysteine peptidase, transcript variant alpha	-3.13
CASP4	NM_001225.3	caspase 4, apoptosis-related cysteine peptidase, transcript variant alpha	-3.08
SOCS4	NM_199421.1	suppressor of cytokine signaling 4, transcript variant 1	-3.04
EIF2AK2	NM_002759.1	eukaryotic translation initiation factor 2-alpha kinase 2, transcript variant 1	-3.03
IFIT2	NM_001547.4	interferon-induced protein with tetratricopeptide repeats 2	-3.00

* SIR, signal intensity ratio

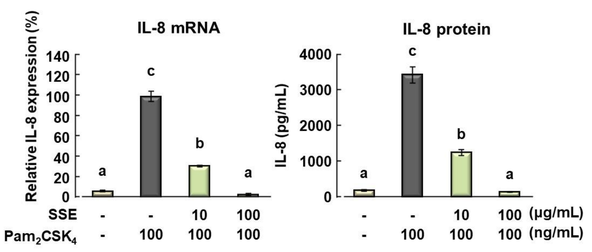
TLR2 expression was downregulated by the largest degree among genes with strong suppression (SIR < -3) .

Step 2



SSE significantly and dose-dependently suppressed TLR2 expression in TNF-α stimulated (A) and unstimulated (B) cells. TLR2 protein expression on the surface of TLR2-positive cells was decreased from 12.8% to 5.0% by SSE treatment (C).

Step 3

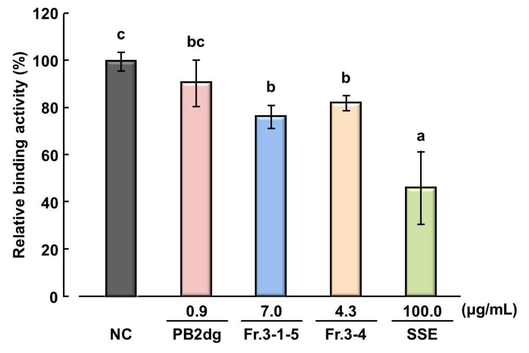


SSE significantly and dose-dependently suppressed IL-8 mRNA and protein expression induced by Pam_2CSK_4.

Step 4

All fractions obtained from SSE suppressed TLR2 ligand-induced IL-8 expression, but only F3 dramatically suppressed TLR2 expression by treatment with relative concentrations of SSE (A and B). The F3-derived fractions, particularly F3-1 and F3-4, suppressed IL-8 expression accompanied by decreased TLR2 expression (C and D). F3-1-5 and F3-4 were also suppressed TLR2 (E) and IL-8 (F) expression in the dose dependent manner. Furthermore, PB2dg isolated from F3-1-5 suppressed IL-8 expression without suppressing TLR2 expression (G).

Step 5

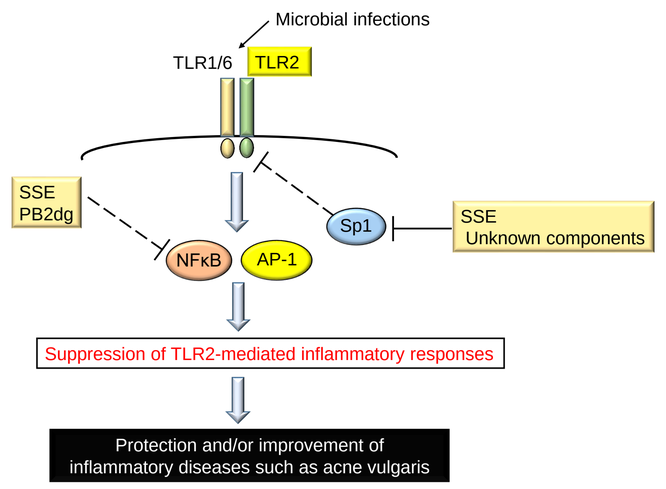


SSE and two fractions significantly suppressed Sp1 binding activity. Conversely, no significant suppression on the activity was found in PB2dg.

Conclusions:

✓ SSE dramatically suppressed TLR2 and TLR2 ligand-induced IL-8 expression in keratinocytes.
✓ PB2dg isolated from SSE, which has been reported to inhibit NF-κB and AP-1 transcriptional activity [5], might be an active component suppressing TLR2-mediated IL-8 production without TLR2 downregulation.
✓ Unknown components contained in SSE might be involved in the inhibitory effect on TLR2 expression via the suppression of Sp1 binding activity.
✓ SSE also inhibited the activity of *C. acnes* lipase, one of the major factors in the acne pathogenesis (data not shown).

SSE may be a useful ingredient to improve inflammatory skin diseases such as acne vulgaris with a different approach than antibiotics and antifungal drugs which are reported to have adverse effects that restrict their use.



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