Kanuka (*Kunzea ericoides*) Leaf Extract suppresses the activity of caspase 1 by NLRP3 inflammasome formation, and is expected to show an effective anti-aging effect.

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Abstract

Background: The skin damages with aging such as wrinkles, spots and dryness are related to inflammatory cytokines secreted by various stimuli. Among various mechanisms of cytokine secretion, IL-1β and IL-18 have unique mechanisms and show rapid reactivity, prodromal IL-1β and IL-18 synthesized by transcription, are processed by various stimuli, and then active IL-1β and IL-18 are secreted. In order to solve skin problems involving inflammatory cytokines, we focused on the mechanism of this rapid reaction and researched on natural products that act at this point. It is the activity of caspase 1 through inflammasome formation regulates this rapid response. Inflammasome is a protein complex that exists in cells. It is activated primarily by bacterial and viral infections, but it has been reported that the NLRP3 inflammasome also can be activated by reactive oxygen species produced intracellularly caused by ultraviolet rays and stress responses. Therefore, we researched natural products that can suppress the activity of caspase-1 induced by ultraviolet rays.

Methods: We prepared Kanuka (*Kunzea ericoides*) Leaf Extract, and evaluated antioxidative effect, supressive effect on caspase-1 activity, anti-inflammatory effect *in vivo*.

Results: It was conformed that Kanuka Leaf Extract has antioxidative effect, supressive effect on caspase-1 in dermal fibroblasts, anti-inflammatory effect on UV-induced erythema *in vivo*.

Conclusion: It is considered that inhibitory effect of Kanuka Leaf Extract on caspase 1 activity exerts an effective anti-inflammatory effect by acting on the early stage of inflammation, and can suppress the dryness and aging of the skin due to inflammation.

Keywords: Kanuka (*Kunzea ericoides*), inflammasome, caspase 1, inflammaging

Introduction.

NLRP3 is one of the NLRP (NACHT leucine-rich repeat protein) family that is pattern-recognition receptors (PRRs) for the pathogens, damaged cells, and environmental components. NLRP3 activated by recognizing PAMPs (pathogen-associated molecular patterns, such as bacterial DNA, RNA), or DAMPs (damage-associated molecular patterns, such as ATP, UV-B). The activation of NLRP3 leads to oligomerization and assembly of NLRP3, ASC (apoptosis-associated speck-like protein containing a caspase-recruitment domain), and pro-caspase-1. This huge protein complex is called NLRP3 inflammasome. Caspase-1 is activated by autocatalytic activation in NLRP3 inflammasome. Activated caspase-1 cleaves and mature the inflammatory cytokines, such as prodromal IL-1β, IL-18. In addition, caspase-1 cleaves gasdermin D to form pores in the plasma membrane. Secretion of active IL-1β, IL-18 cause various inflammatory phenomenon in the tissue, and gasdermin pore induce the programed cell death known as pyroptosis [1]. The skin is constantly exposed to ultraviolet rays (UV-B) which is known as non-pathogenic NLRP3 inflammasome activated factor. Recently, it has been reported that inflammasome is activated by UV-B irradiation and is secreted IL-1β in keratinocyte [2]. Furthermore, the downstream of IL-1α/β receptor has the secretion of the cytokines, such as IL-6, IL-8. IL6/8 is well-known as SASP (Senescence-Associated Secretory Phenotype), which promote cellular senescence [3]. Typically, the aging arrests the cell proliferation and decrease various metabolic functions. Because of this, the activation of NLRP3 inflammasome accelerates cellular senescence in the skin. In addition, the secreted IL-1β by NLRP3 inflammasome in skin causes the decreasing of fillagrin-2 (FLG-2), which moisturize the skin [4]. From these reports, it is suggested that the activation of NLRP3 inflammasome by UV-B exposure accelerates the skin aging and skin dryness.

Our reports show that UV-B irradiation induced the activation of inflammasome in human dermal fibroblasts. Surprisingly, Kanuka Leaf Extract can suppress caspase-1 activity by UV-B induced NLRP3 inflammasome activation in fibroblasts.

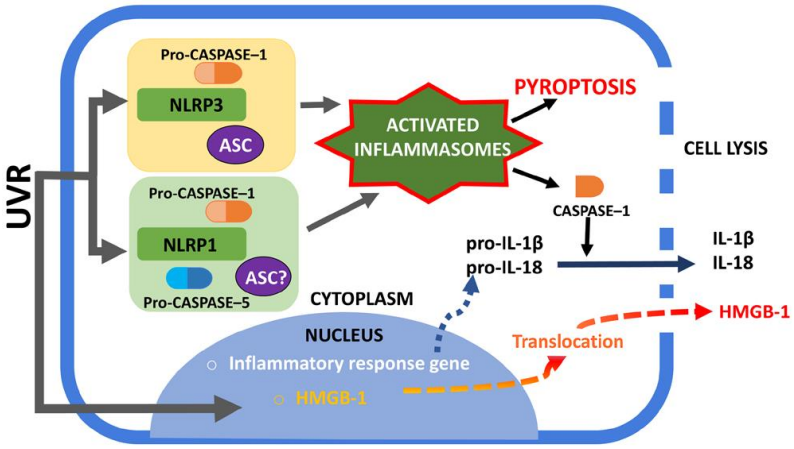

Fig.1 Activation of NLRP1 and NLRP3 inflammasomes [5]

About Kanuka

Kanuka (*Kunzea ericoides*), commonly known as “white tea tree”, is a traditional medicinal plant endemic to New Zealand (Fig.2). Although it is a low tree, sometimes it can grow up to a height of 20 to 30 m, and small white flowers blooming on the top of branches like a piece of brocade from September to the following February. The Maori, New Zealand's indigenous, use its leaves as medicine, and kanuka honey is also a popular health food.

Fig.2 The photograph of Kanuka Leaf

Materials and Methods.

Preparation of Kanuka Leaf Extract

Extraction was prepared from dried Kanuka leaves using a polar solvent, and then the mixture was filtered and purified to prepare Kanuka Leaf Extract.

INCI name: Kunzea Ericoides Leaf Extract

Component analysis of Kanuka Leaf Extract

Kanuka Leaf Extract was analyzed by using HPLC. The conditions are shown in Fig. 3. Many peaks that seemed to be polyphenols could be confirmed. A particularly large peak was found to be chlorogenic acid (Fig.3).

Method
Column：C18　15 cm × 4.6 ID
Colum Temp.：40 °C
Sample Temp.：4 °C
UV Detection：254 nm

Gradient			
Time	Flow rate	Mobile phase A 0.1 % formic acid aqueous solution	Mobile phase B Acetonitrile
0 min	1 ml/min	100 %	0 %
3 min		100 %	0 %
45 min		5 %	95 %
50 min		5 %	95 %
55 min		100 %	0 %

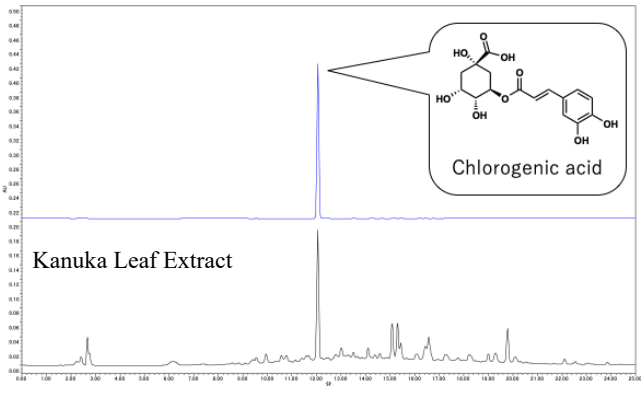

Fig.3 Component analysis of Kanuka Leaf Extract

DPPH radical scavenging assay

A mixture of DPPH solution, ethanol and acetic acid-sodium acetate buffer (pH5.5) was added to each extract. After standing at 37℃ for 20 minutes, the absorbance at 550 nm of the reaction

solution was measured to calculate the residual amount of DPPH radicals.

Caspase-1 assay

Normal human dermal fibroblasts were seeded on 24-well plates, and each extract were added after culturing for 1 day. After further culturing for 1 day, culture medium was replaced to the medium containing LPS at 1.0 μg/mL without phenol red and cells were cultured for 1 hour. Afterwards, cells were irradiated UV-B of 100 mJ/cm2 for caspase-1 induction. After 4 hours, the cells were stained with Pyroptosis/Caspase-1 Assay Green (Immunochemistry Technologies, LLC, USA) to detect the caspase-1 activity in the cells. The cells were detached with trypsin, and then inactivated trypsin with ice-cold 5% FBS-containing PBS. After cells were rinsed twice with ice-cold PBS containing 0.1% BSA, analyzed by flow cytometer (BD Accuri™ C6 Plus, BD Biosciences) to calculate the average of fluorescence intensity (Ex. = 488nm, Em. = 538nm).

Suppression test for erythema formation by ultraviolet rays

The lotion containing 0.5% Kanuka Leaf Extract and control lotion were applied to each test area in forearm of 6 subjects twice a day for a week.

After the application is finished, the erythema index of each test area was measured (initial value) by MEXAMETER (Courage + Khazaka electronic GmbH). After that, UV-B corresponding to 1 MED of each subject was irradiated to each test area.

The next day, each test area was photographed and the erythema index was measured same as measuring initial value.

Results.

DPPH radical scavenging assay

It was confirmed that Kanuka Leaf Extract can eliminate effectively DPPH radical in a concentration-dependent manner.

Evaluation of activity of caspase-1

The activity of caspase 1 through inflammasome formation in dermal fibroblasts was enhanced by UV-B irradiation. Compared with the UV-B Irradiation Control, the suppression was observed in Kanuka Leaf Extract added group in a concentration-dependent manner. And a significant inhibitory effect was especially confirmed in the group added with 2% Kanuka Leaf Extract.

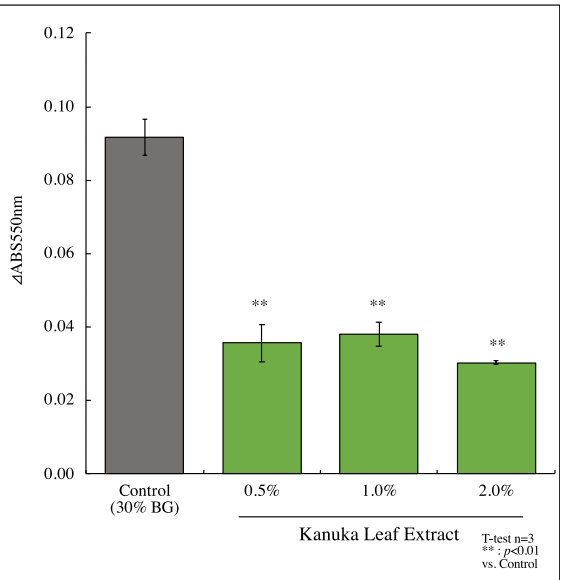

Fig.4 DPPH radical scavenge activity of Kanuka Leaf Extract

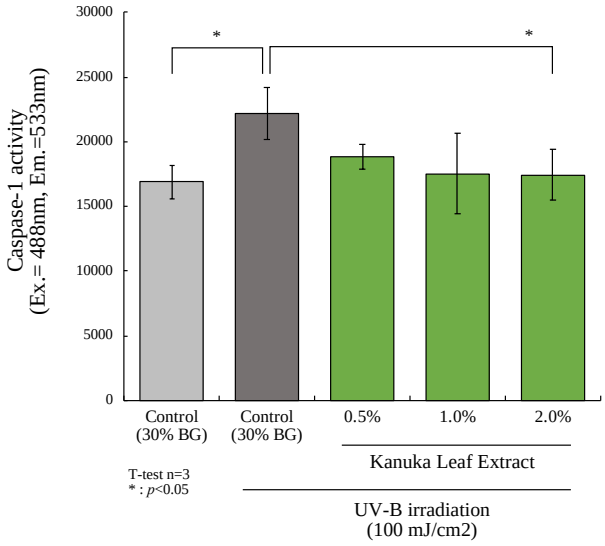

Fig.5 Supressive effect of Kanuka Leaf Extract on caspase-1 activity

Activity of intracellular caspase-1 cultured with Kanuka Leaf Extract in 2 days, was measured 4 hours after UV-B irradiation.

Suppression test for erythema formation by ultraviolet rays

UV-B was irradiated to the test areas on which the lotion containing 0.5% Kanuka Leaf Extract or the control lotion were applied twice a day for a week. The next day, erythema was confirmed in each subject. The degree of erythema was compared by calculating the ratio of changes in the erythema index of each test area before and after UV-B irradiation.

Then, it was found that the value of the lotion-applied area containing 0.5% Kanuka Leaf Extract was statistically significantly lower than the increase ratio of the erythema of the control lotion-applied area (Fig. 6).

Figure 7 shows a typical test area. It was shown that the degree of erythema in the lotion-applied area containing 0.5% Kanuka Leaf Extract was low compared with control lotion applied area.

Discussion.

It has been reported that IL-1β and IL-18, which are cleaved and secreted by caspase-1 through NLRP3 inflammasome. These cytokines suppress the expression level of filaggrin-2, which is known as one of natural moisturizing factor. Therefore, it is suggested that NLRP3 inflammasome cause and accelerate the skin dryness. On the other hand, IL-1β is one of the SASPs (Senescence Associated Secretory phenotype), which has a great impact on skin aging such as wrinkles, and induce tissue destruction by activated neutrophils. In addition, it is known that the cytokines such as IL-6 and 8 are produced at the downstream of IL-1α/β receptor signaling. IL-6 and 8 are also the components of SASPs. These SASPs promote the aging of around cells in the skin. And it is well-known that IL-18 promote interferon gamma (INFγ) which is important activator of macrophage. Our reports show that caspase-1 activated by UV-B

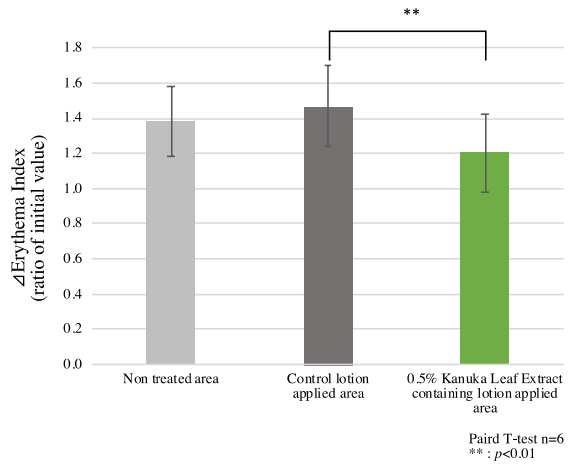

Fig.6 Change of erythema index by UV-B irradiation

Each sample was applied twice a day for a week before UV-B irradiation.
Erythema index were measured and photos were taken 1 day after UV-B irradiation.

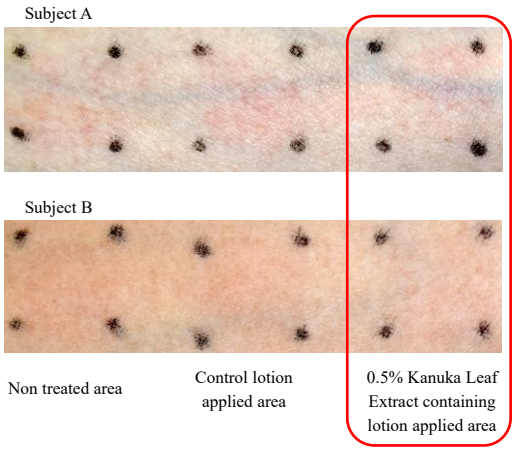

Fig.7 The photograph of typical test area 1 day after UV-B irradiation

irradiation through activation of NLRP3 inflammasome in human fibroblasts. This fact suggests that UV light causes inflammation of the NLRP3 inflammasome in the dermis area. And Kanuka Leaf Extract are not only a very strong antioxidant, but also suppresser for caspase-1 activation. It is considered that the caspase-1 activity inhibitory effect of Kanuka Leaf Extract exerts an effective anti-inflammatory effect by acting on the early stage of inflammation, and can suppress the dryness and aging of the skin due to inflammation(inflammaging).

Conclusion.

We have confirmed that there is inflammasome-related inflammation in dermal fibroblasts as well. Then, we found Kanuka Leaf Extract as a natural functional ingredient that can suppress it and prevent skin aging(inflammaging).

Conflict of Interest Statement.

NONE.

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