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The Path to More Attractive Male Skin: Development of a More Effective Whitening Agent for Men's Pigmented Spots

KOBAYASHI

Kubo, Hirokazu*; Kobayashi, Soko; Tsunematsu, Nozomi; Nakashiro, Yota; Yoshida, Ikuyo; Kuriyama, Ken-ichi;
Healthcare Division, KOBAYASHI Pharmaceutical Co., Ltd., Osaka, Japan.

Introduction:

A previous study that we conducted on solar lentigines in men yielded the following results [1] :

1) the brightness difference (ΔL) between areas with and without solar lentigines was significantly greater in men than in women, indicating that they tend to be conspicuous (Fig. 1)
2) solar lentigo areas in men had a higher activity of plasmin, an inflammatory enzyme that promotes melanin production, compared to areas without solar lentigines (Fig. 2)
3) men with fewer and lighter pigmented spots were judged to be more attractive by women (Fig. 3)
4) tranexamic acid (TXA), an antiplasmin agent, was effective against pigmented spots in men

Based on these findings [1], the development of a more effective formulation was required to improve the large and dark pigmented spots in men.

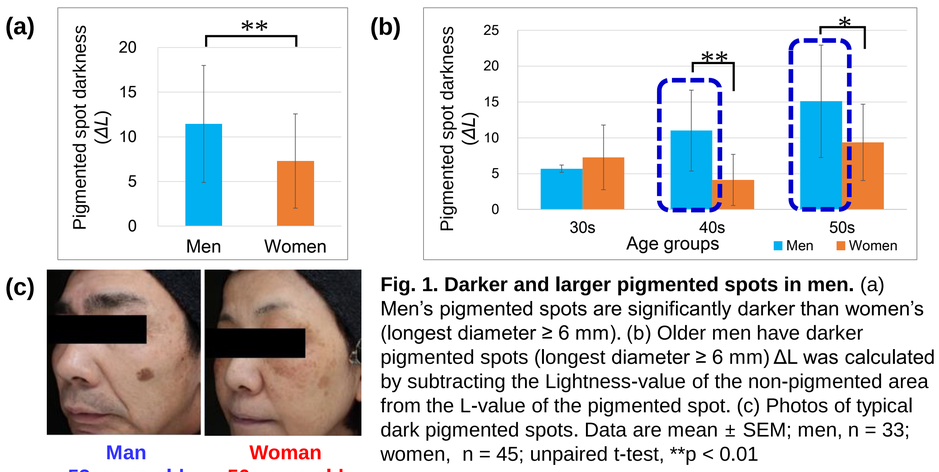

Fig. 1. Darker and larger pigmented spots in men. (a) Men's pigmented spots are significantly darker than women's (longest diameter ≥ 6 mm). (b) Older men have darker pigmented spots (longest diameter ≥ 6 mm) ΔL was calculated by subtracting the Lightness-value of the non-pigmented area from the L-value of the pigmented spot. (c) Photos of typical dark pigmented spots. Data are mean ± SEM; men, n = 33; women, n = 45; unpaired t-test, **p < 0.01

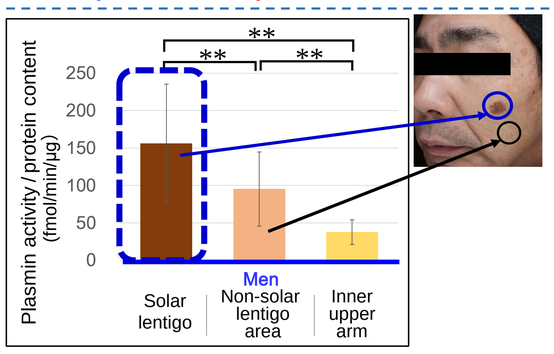

Fig. 2. Men's solar lentigo showed higher plasmin activity.
Men with solar lentigo on the cheek. n = 16, Data are mean ± SEM; paired t-test, **p < 0.01.

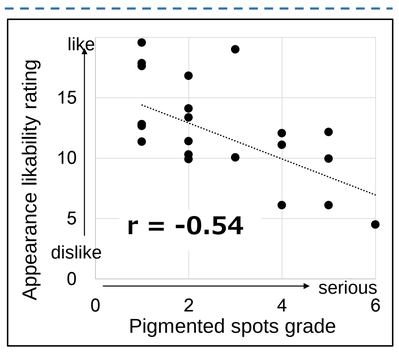

Fig. 3. Pigmented spots have an effect on impression. Men in their 40s (n = 20); women (n = 20); Pearman's rank correlation coefficient, p<0.01.

Aim of this study

1) **To search for plant extracts with an anti-plasmin activity**
2) **To identify plant extracts with an anti-plasmin activity that work with TXA synergistically to inhibit melanogenesis**
3) **Development of preparations that are more effective in treating the larger and darker pigmented spots in men**

Materials & Methods:

1) Assay of plasmin inhibitory activity of plant extracts

The plasmin-like activity was measured using a substrate (BOC-VAL-LEU-LYS-MCA) [2]. An EnSight Multimode Plate Reader (PerkinElmer, USA) was used to measure the fluorescence of substrate-derived 7-amino-4-methylcoumarin (MCA) at Ex 380 nm and Em 460 nm. Plant extracts provided by ICHIMARU PHARCOS Co., Ltd., Japan, and MARUZEN PHARMACEUTICALS Co., Ltd., Japan, were used. See Full paper (Table 1) for details.

2) Evaluating melanin production using a 3D-cultured skin model

The 3D-cultured skin model (MEL-300-A, Kurabo Industries Ltd., Japan) was cultured for 12 days, with UV-B irradiation, medium replacement, and the addition of PBS containing the whitening agents conducted every other day. Melanin contents were measured using an absorbance meter (MULTISKAN FC, Thermo Fisher Scientific, USA) at 405 nm.

3) Clinical study

Healthy Japanese volunteers, 17 men aged 33-55, applied 2% Tranexamic acid (TXA) cream twice-daily on the solar lentigo on the cheek for 6 weeks. This cream contained: 2% TXA, 0.5% SBE, 0.5% CLE, 10% emulsifier, 30% liquid and solid oil, 10% moisturizing agent, moderate amounts of preservatives/fragrance, and water to reach 100% volume. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Results & Discussion:

Result 1 7 plant extracts inhibit plasmin activity.

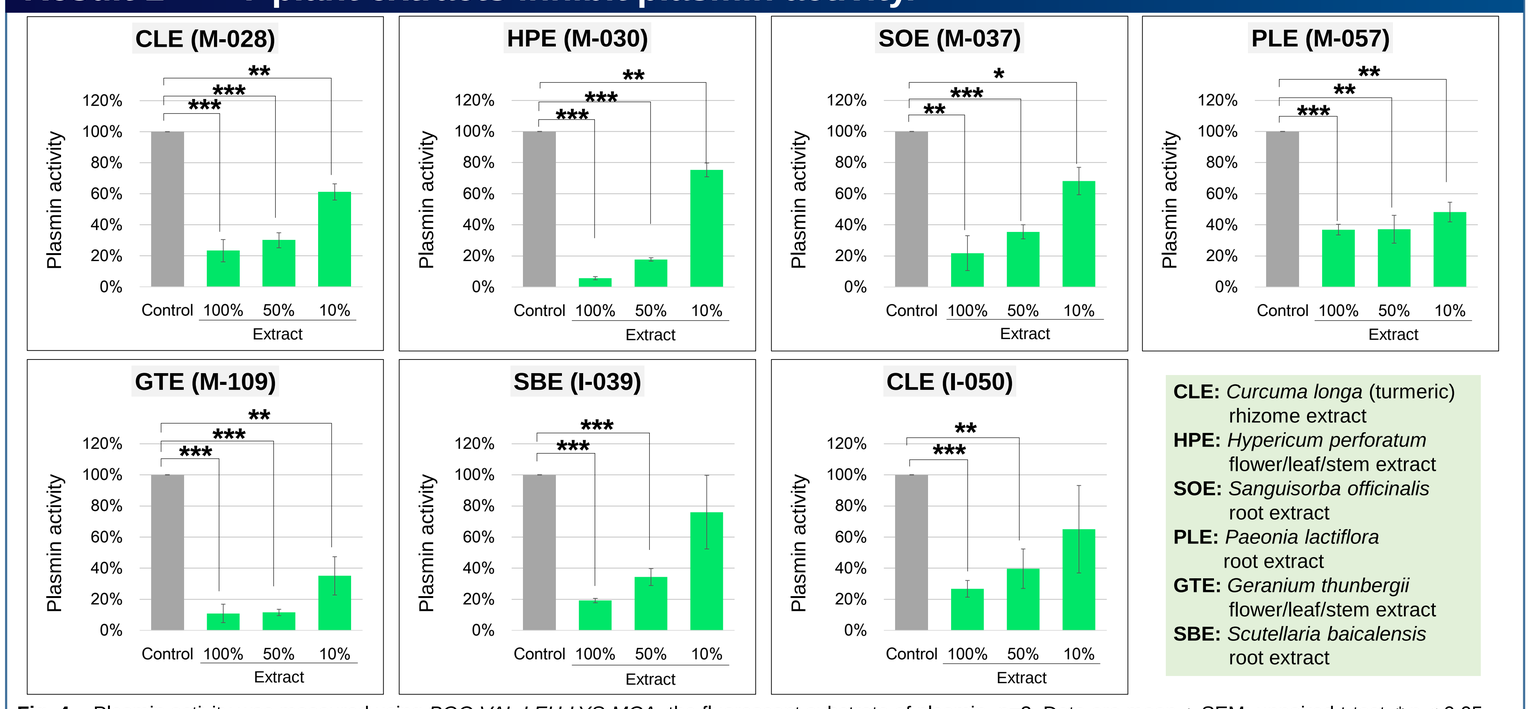

Fig. 4. Plasmin activity was measured using *BOC-VAL-LEU-LYS-MCA*, the fluorescent substrate of plasmin. n=3, Data are mean ± SEM; unpaired t-test, * p < 0.05, ** p < 0.01, *** p < 0.001

Result 2 SBE and CLE increased the effectiveness of TXA against the melanin production.

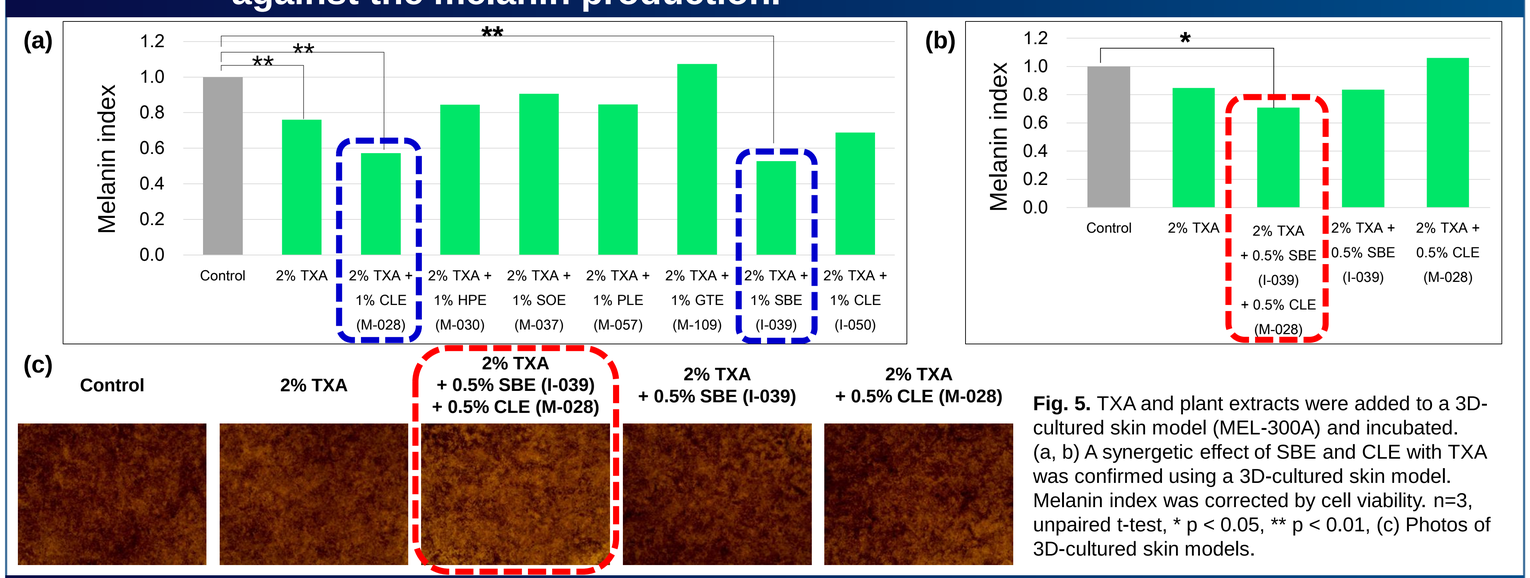

Fig. 5. TXA and plant extracts were added to a 3D-cultured skin model (MEL-300A) and incubated. (a, b) A synergetic effect of SBE and CLE with TXA was confirmed using a 3D-cultured skin model. Melanin index was corrected by cell viability. n=3, unpaired t-test, * p < 0.05, ** p < 0.01, (c) Photos of 3D-cultured skin models.

Result 3 The cream containing TXA, SBE, and CLE showed a whitening effect on men's solar lentigo

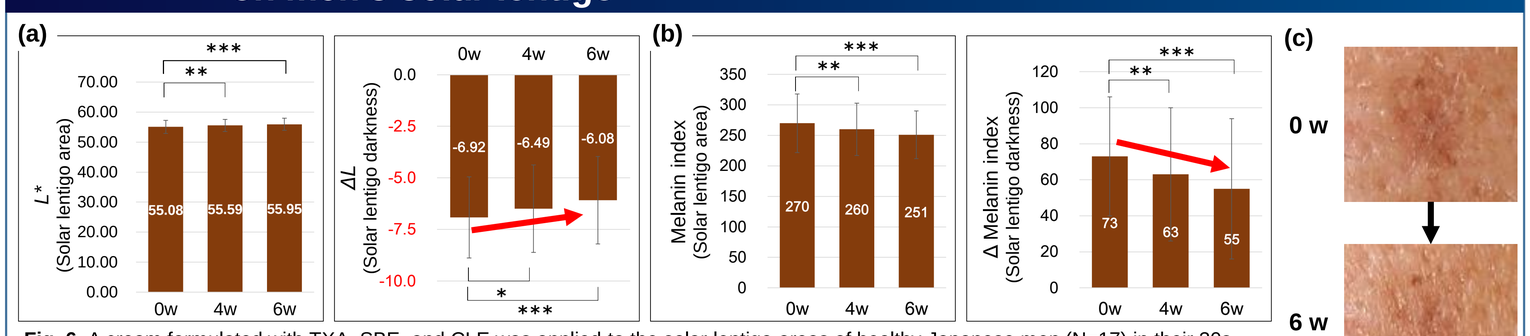

Fig. 6. A cream formulated with TXA, SBE, and CLE was applied to the solar lentigo areas of healthy Japanese men (N=17) in their 30s to 60s, and the *L** values before and after the cream application were measured. (a) *L**, (b) Melanin index, *ΔL* and Δ Melanin index were obtained by subtracting the value of *L**, Melanin index (without solar lentigo area) from *L**, Melanin index (solar lentigo area). (c) Photos of typical pigmented spots, Data are mean±SD, paired t-test, * p < 0.025, ** p < 0.005, *** p < 0.0005

Discussion

SBE and CLE showed antiplasmin activity (Fig. 4) and synergetic effect with TXA, suggesting that they may inhibit melanogenesis by controlling the expression of α-MSH and other factors stimulated by plasmin activity (Fig. 7) [3]. However, further studies are needed to identify the active ingredients in SBE and CLE and elucidate their mechanisms of activity.

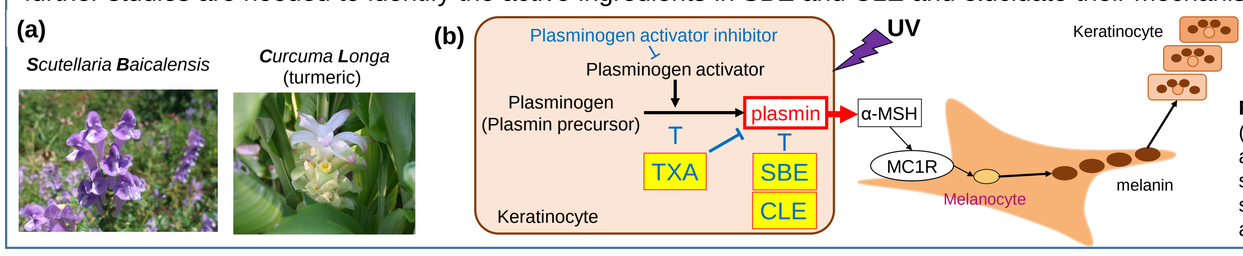

Fig. 7.
(a) Plants with anti-plasmin and whitening effects in this study, (b) Schematic diagram showing the mechanism of action of SBE and CLE

References:

1. Kobayashi S, et al., (2018), Why are Pigmented Spots Darker and Larger in Men?, The IFSCC Congress
2. Homma R et al., (2005), Modulation of blood coagulation and fibrinolysis by polyamines in the oresence of glycosaminoglycans, Int. J. Biochem. Cell Biol. 37: 1911-1920
3. Ando H, et al., (2010), Quasi-Drugs Developed in Japan for the Prevention or Treatment of Hyperpigmentary Disorders, Int. J. Mo. Sci. 11: 6, 2566-2575

Conclusions:

- SBE and CLE were found to have antiplasmin activity.
- SBE and CLE increased the effectiveness of TXA against melanogenesis in a 3D-cultured skin model.
- The combination of TXA, SBE and CLE showed a whitening effect in men's solar lentigo.