

Squamometry: an evaluation method for a barrier protectant (tannic acid)

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Squamometry for evaluating skin barrier substances is described. Forearms of 8 volunteers were dosed with 0, 0.25, 0.5, and 1% sodium lauryl sulfate (SLS) in distilled water (semi-open method) for 24 h, to skin pretreated with distilled water and 5% tannic acid, a model barrier protectant. Squamometric evaluation indicated the skin damage increased with SLS concentration in a dose-dependent manner, and that tannic acid reduced the damage ($p < 0.01$). The results suggest that squamometry may be a useful method for determining efficacy of skin barrier substances.

Key words: squamometry; sodium lauryl sulfate; tannic acid; skin barrier substance; prevention.
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In vitro (1, 2), animal (3, 4) and human methods (2, 5–9) for evaluating barrier products against irritation exist. Such methods should be convenient, reproducible and clinically relevant. Squamometry assesses irritant potential of detergents and cosmetics (10, 11).

This study documents the skin-barrier efficacy of tannic acid with squamometry. Tannic acid and tannins have been used as a topical agent for eczematous skin and burn injuries. Their effect is possibly by virtue of their astringency (12). Takizawa et al. (13) showed that the tannin extracted from persimmon prevented skin from becoming chapped. This damage may be produced by dishwashing detergent and they suggested that tannin formed a protective membrane on skin. Tannic acid also inhibited skin damage induced by a surfactant, linear alkyl benzene sulfonate, in our laboratory (unpublished data*).

Materials and Methods

Subjects

8 healthy Caucasian volunteers (6 female and 2 male, age 45–68, 50 ± 8 years) participated after providing informed consent. The Institutional Review Board was from University of California San Francisco Medical Center.

Materials

Sodium lauryl sulfate (95% purity) (SLS, Sigma, St. Louis, MO) was dissolved in distilled water and the following concentrations (W/W) prepared: 0% (distilled water), 0.25%, 0.5%, 1% tannic acid (Dainihon-seiyaku, Osaka, Japan) at 5% (W/W) was dissolved in distilled water.

Procedure

The volar side of one forearm was divided into 8 sites of 1.5 cm². 10 μ l of tannic acid was applied

* Protective effect of tannic acid solution was evaluated using a 24-h patch test with 5% linear alkyl benzene sulfonate (LAS) on human forearm. Tannic acid solution and control solution were applied 30 min before LAS application. Visual change was scored with ESCD simple scoring system; visual score (average $\pm$ sem, $n=8$):

	24 h after patch removal	48 h after patch removal
control	1.2 ± 0.4	1.2 ± 0.4
tannic acid	0.4 ± 0.1 ($p < 0.05$)	0.2 ± 0.1 ($p < 0.05$)

Statistics: Wilcoxon signed rank test (control versus tannic acid).

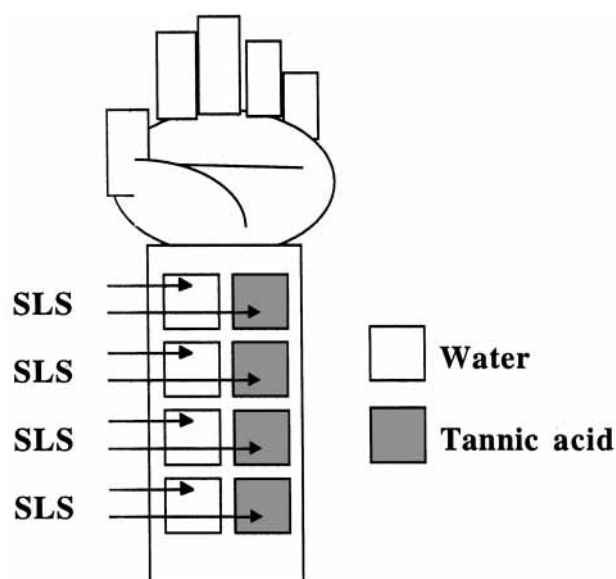


Fig. 1. Sample application to the forearms.

open to 4 sites and distilled water was applied as a control in the other 4 sites. 30 min after this pre-treatment, 10 μ l of SLS solution was applied open. SLS solutions of the same concentration were applied side by side (Fig. 1). These test sites were covered by cotton and paper tape 30 min after SLS application (Semi-open application) (14). 24 h after application, the covering materials were removed and the test sites rinsed, but not scrubbed, with running tap water, and patted dry, followed by air drying for 30 min (20°C) before stratum corneum was collected.

Sticky discs (D-Squames®, Cuderm Corporation, Dallas, TX) were placed on the test sites

with standard pressure (110–150 g/cm²) and each site was stripped 2 $\times$. The collected disks were stained with PMS stain (Delasco, Council Bluffs, IA) and the color measured with a Chromameter CR-300 (Minolta, Osaka, Japan) on $L^*a^*b^*$ system. The value $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ was used as an index of skin damage (10, 11).

Statistics

Statistical analysis of the data between SLS concentrations at the same pre-treatment sites was performed with Fisher's PLSD method; comparison of the data between pre-treatment of water and tannic acid at same SLS concentration was performed with the paired *t*-test.

Results and Discussion

Results are shown in Fig. 2.

The C^* value increased linearly with increasing SLS concentration in water pre-treatment as well as with tannic acid pretreatment sites in both the 1st and 2nd stripped stratum corneum (SC). In the 1st and 2nd stripped SC at the 1% SLS site and 1st stripped SC of the 0.5% SLS site, the C^* value in tannic acid-treated sites was significantly decreased compared with water-treated sites. Thus tannic acid reduced SLS-induced damage.

Squamometry has been studied as a method for accessing potential irritancy of cleansing products to SC (10, 11, 15, 16). The C^* value, a parameter for evaluation in squamometry, reflects the intensity of dye binding to corneocytes and the amount of corneocytes collected by the adhesive disc. (15) Dye binding increases as skin damage become severe,

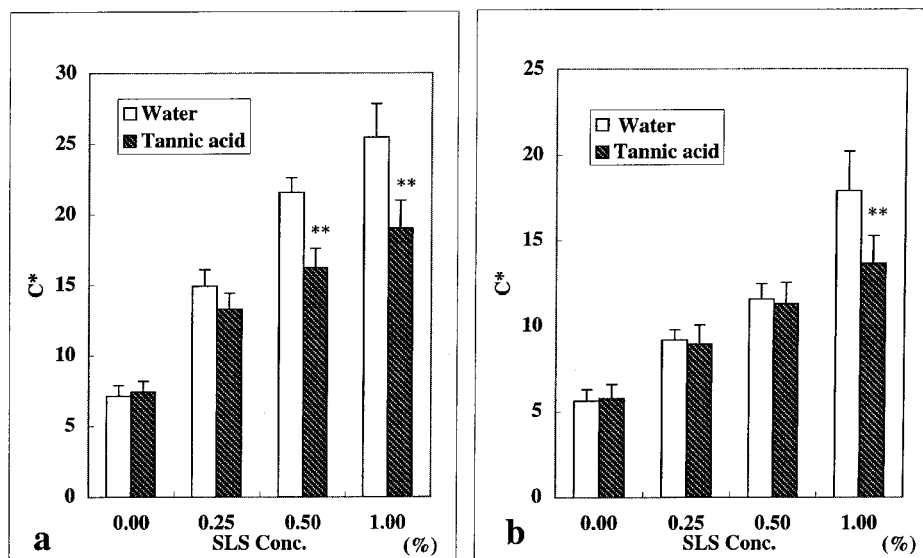


Fig. 2. The C^* values of 1st stripped SC(a), and 2nd stripped SC(b). The results are mean $\pm$ SE. Significant differences between the water application group and the tannic application group at same SLS concentrations are indicated by ** ($p < 0.01$).

while the amount of collected corneocytes decreases due to inter-corneocyte loosening (15). Therefore, C^* value in squamometry would not correlate with the damage of the skin or to other skin properties, such as the TEWL value in some cases, especially when skin damage is severe. However, in this experiment, squamometry clearly defined the damage to the skin and the skin barrier efficacy of tannic acid, which was observed to have a skin barrier effect against a surfactant. Thus, squamometry may be a suitable method for evaluating the efficacy of barrier substances when minimally damaged conditions are chosen, for example, as with the semi-open application used here.

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