Anti-allergic Effect of Tea-Leaf Saponin (TLS) from Tea Leaves
(Camellia sinensis var. sinensis)

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We investigated the anti-allergic effect of tea-leaf saponin (TLS), which was a mixture of saponins separated from the leaves of Camellia sinensis var. sinensis, in guinea pigs and rats. TLS (20—100 mg/kg) dose-dependently inhibited experimentally-induced asthma, and ID₅₀ was 61.7 mg/kg. TLS (20—100 mg/kg) dose-dependently inhibited a 48 h homologous PCA (passive cutaneous anaphylaxis) reaction, and the inhibitory effect was similar to that of tranilast. TLS (1—100 µg/ml) also inhibited the release of antigen-induced leukotriene (LT) C₄ from sensitized guinea pig lung samples in a dose-dependent fashion, but did not prevent histamine release. TLS (0.01—0.5 µg/ml) inhibited histamine release from rat peritoneal mast cells induced by compound 48/80. At higher concentrations, TLS elicited histamine release. These findings suggest that TLS may be a useful protective agent against clinical allergy, and that the inhibitory effects of TLS on mediator release are in some way related to its inhibitory effect on experimentally-induced asthma and PCA reaction.

Keywords tea-leaf saponin; Camellia sinensis var. sinensis; anti-allergic effect

It has been reported that the catechins from tea inhibit the formation of lipid peroxidation induced by ADP and NADPH in microsomes of the liver. Tannins are the major components of an extract from tea, and they have been shown to exhibit various biological and pharmacological activities. Saponins from the plant have been known to be cardiotonic, diuretic, hypoglycemic, expectorant and anti-inflammatory. The constituents of saponins from the leaves of Camellia sinensis var. sinensis (tea-leaf saponins) were reported by Hashizume. Recently, a major saponin, from tea leaves, was separated and the structure was elucidated. In this report, we describe the anti-asthma effect of tea-leaf saponin (TLS), which is a mixture of saponins separated from the leaves of Camellia sinensis var. sinensis.

MATERIALS AND METHODS

Materials Male Hartley guinea pigs weighing 450—500 g and male Wistar rats weighing 250—300 g were used. TLS from tea leaves (C. sinensis var. sinensis), which was a mixture of teasaponin B₁—B₄, was supplied from Ito-en Co., Ltd. The other drugs and chemicals were purchased from commercial sources.

Methods Guinea pigs were sensitized according to the method of Mota. Ovalbumin was used as an antigen. Two weeks later, the animals were anesthetized with an intraperitoneal injection of pentobarbital (30 mg/kg) and a cannula was inserted into the trachea. The animals were then paralyzed by an intravenous injection of gallamine triethiodide (1 mg/kg) and were artificially ventilated by means of a respiratory pump (Matsushita, AP-TLC) connected to the tracheal cannula (stroke volume 10 ml, 70 strokes/min). The airway pressure was measured by means of a pressure transducer (Nihon Koden, SCK-580) connected to a side arm of a tracheal cannula. The test drugs were administered orally 1 h before the antigen challenge.

Male BN rats (Charles River Japan Inc.) weighing 200—250 g were immunized according to the method described previously, and antisera were prepared. A conjugate of 2,4,6-trinitrobenzene sulfonic acid linked bovine serum albumin (TNP-BSA) was used as an antigen, and its antiserum titer was determined in relation to a 48 h homologous PCA (passive cutaneous anaphylaxis). The diluted (1:80) antiserum were intradermally injected into the back skin of normal deplated rats. After 48 h, 0.5 mg of TNP-BSA dissolved in 0.5 ml of 0.5% Evans blue solution was injected intravenously, and the animals were exsanguinated 30 min later. The blue areas were measured. Test drugs were orally administered 1 h before the antigen challenge.

Histamine and leukotriene (LT) C₄/D₄ release from lung pieces of sensitized guinea pigs were measured as described previously. Histamine release was measured by means of a fluorometric assay. The amount of LTC₄/D₄ was measured using a radioimmunoassay kit (Amershams). Histamine release from rat peritoneal mast cells was measured as described previously. Peritoneal mast cells were collected from the abdominal cavity of rats, and purified to a level higher than 95% by Percoll density gradient centrifugation. The mast cells were preincubated with various concentrations of TLS for 15 min at 37°C, and compound 48/80 (48/80) (0.5 µg/ml) was added and the incubation was continued for 10 min at 37°C. The histamine released in the supernatant and the residual histamine were determined by means of a fluorometric assay. Histamine release was expressed as a percentage of total histamine. The results are reported as the means ± standard error of the means (S.E.), unless otherwise indicates.

Statistical significance was evaluated by the unpaired Student's t-test, with p < 0.05 being regarded as significant. ID₅₀ was calculated according to the method of

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Litchfield-Wilcoxon.

RESULTS

The effect of TLS on antigen-induced bronchospasms in actively sensitized guinea pigs was compared with that of tranilast. TLS (20—100 mg/kg, p.o.) inhibited the antigen-induced increase in the airway pressure in a dose-dependent fashion (Fig. 1), and ID₅₀ was 61.7 (36.7—103.7) mg/kg. The effect of tranilast corresponded to approximately 3/4 that of TLS. The effect of TLS on the homologous PCA reaction in rats was compared with that of tranilast. In the control group, the amount of dye leakage was 14.2±1.47 μg/site. As shown in Fig. 2, both TLS and tranilast dose-dependently inhibited the PCA reaction and no significant difference was observed. The intensity of the inhibitory effect of TLS on the PCA reaction was less than that on the antigen-induced increase in airway resistance. When the lung pieces from sensitized guinea pigs were exposed to the antigen, 24.5±0.5% histamine and 82.47±0.47 ng/g tissue LTC₄/D₄ were released into the medium. As shown in Fig. 3, TLS inhibited LTC₄/D₄ release in a dose-dependent fashion, but did not inhibit histamine release (data not shown). When rat peritoneal mast cells were exposed to 48/80 (0.5 μg/ml), histamine was released at 38.6±5.5% of the total histamine content. TLS (0.01—0.5 μg/ml) did not elicit histamine release, and the pretreatment with TLS inhibited 48/80-induced histamine release in a dose-dependent fashion, but 1 μg/ml TLS actually elicited

![Graph A](image1)

Fig. 1. Inhibitory Effects of TLS Isolated from the Leaves of *Camellia sinensis* var. *sinensis* and Tranilast on the Antigen-Induced Increase in Airway Resistance in Actively Sensitized Guinea Pigs

- (A) Time-course of changes of tracheal pressure: (○) control; (●) 20 mg/kg TLS; (▲) 50 mg/kg TLS; (△) 100 mg/kg TLS; (■) 100 mg/kg tranilast. Each value represents the mean ± S.E. of the % change in the antigen-induced increase of airway resistance against the control level before antigen challenge (n = 5). Significantly different from the corresponding control, *p < 0.05, **p < 0.01.
- (B) % inhibition of TLS and tranilast. Each column represents the mean ± S.E. of the % inhibition at 6 min of (A).

![Graph B](image2)

Fig. 2. Inhibitory Effects of TLS and Tranilast on Rat 48 h Homologous PCA Reaction

- Hatched column: TLS; dotted column: tranilast. Each column represents the mean ± S.E. of the % inhibition against the corresponding control (n = 5).
histamine release (Fig. 4).

DISCUSSION

The oral administration of various extracts from green tea slightly reduced the elevation of serum free fatty acids, lipid peroxides, glutamic pyruvic transaminase (GPT) and liver triglycerides in peroxidized corn oil-fed rats.\(^1\)\(^-\)\(^2\) (-)-Epigallocatechin gallate, a catechin of tea, scavenged the superoxide anion radicals generated in the xanthine-xanthine oxidase system.\(^3\) It has been known that saponins are one constituent in tea leaves. However, the pharmacological activities of TLS had not yet been examined, because of the difficulty of isolation. We believe that this is the first report of its pharmacological study, in vivo.

TLS inhibited both the antigen-induced bronchospasm in sensitized guinea pigs. (Fig. 1) and PCA reaction in rats, dose-dependently. To clarify the mechanisms responsible for the inhibition of antigen-induced bronchoconstriction and PCA reaction, we measured its effects on the release of histamine and LTC\(_4\)/D\(_4\) from lung pieces of sensitized guinea pigs, as well as histamine release from rat peritoneal mast cells induced by 48/80. TLS inhibited antigen-induced LTC\(_4\)/D\(_4\) release from lung pieces in a dose-dependent fashion, but not significantly (Fig. 3). However, TLS did not inhibit histamine release from lung pieces. TLS significantly inhibited the LTD\(_4\)-induced contraction of isolated guinea pig ileum in a dose-dependent fashion.\(^4\)\(^-\)\(^5\) In the preliminary experiment, TLS inhibited the LTD\(_4\)-induced contraction of isolated guinea pig trachea in a dose-dependent fashion, but did not inhibit the histamine-induced contraction. These findings suggest that the inhibitory effects of TLS on LTC\(_4\)/D\(_4\) release and the LTD\(_4\)-induced contraction of the ileum and trachea may be responsible for its anti-allergic effect, though further studies are needed.

Glycyrrhizin, a typical saponin from the radix of *Glycyrrhiza glabra* (*Leguminosae*), has been well investigated. Glycyrrhizin has glucocorticoid-like, estrogen-like, anti-tussive and anti-allergic properties.\(^6\)\(^-\)\(^7\) Saponins are surface active and enhance haemolysis at concentrations higher than a critical micelle concentration (CMC).\(^8\) However, a surfactant is known to be a membrane stabilizer at lower concentrations than CMC, and to be an inhibitor of hypotonic haemolysis and histamine release from rat peritoneal mast cells.\(^9\) One of the important mechanisms of TLS on the release of chemical mediators can be ascribed to the act of membrane stabilization on the lipid bilayer of the plasma membrane.

In the present study, we demonstrated that TLS efficiently inhibits the antigen-induced increase in airway resistance and PCA reaction. Further study to examine whether TLS may be useful as a therapeutic tool in the allergy is in progress in our laboratory. In addition, the possibility that TLS is a useful protective agent against clinical allergy warrants further attention.

REFERENCES