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Inhibitory Effects of Tannins on Hyaluronidase Activation and on the Degranulation from Rat Mesentery Mast Cells[1)]

Hisao Kakegawa,[a] Hitoshi Matsumoto,[a] Koichi Endo,[a]
Toshio Satoh,*,[a] Gen-ichiro Nonaka,[b]
and Itsuo Nishioka[b]

School of Pharmacy, Tokushima University of Arts and Science,[a] Yamashiro-cho, Tokushima 770, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,[b] 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

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The inhibitory effects of tannins on the activation of inactive hyaluronidase and on the degranulation from rat mesentery mast cells induced by compound 48/80 were investigated. Among the tested tannins, 1,2,3,4,6-penta-*O*-galloylglucose, 1,2,3,6-tetra-*O*-galloylglucose (hydrolyzable tannins) and 3,3′-di-*O*-galloylprodelphinidin B-2 (condensed tannins) showed the strongest inhibitory effects in both cases. These results suggest that many tannins may possess anti-inflammatory activity and anti-allergic activity.

Keywords——tannin; hyaluronidase; anti-hyaluronidase activity; degranulation; anti-allergic activity

Tannins are natural products which are believed to have many physiological activities in various kinds of folk medicines. Nishioka *et al.* reported[2-5)] that rhatannin and 1,2,3,4,6-penta-*O*-galloylglucose showed decreasing effects on blood urea nitrogen (BUN), and Okuda *et al.* reported[6)] that many tannins had inhibitory effects on plasmin, mutagenesis and cultured KB cells. Furthermore, (+)-catechin, one of the condensed tannins, showed excellent preventive effects against acute liver injury induced by carbon tetrachloride (CCl_4) and 1-naphthylisothiocyanate (ANIT) in mice and rats.[7-10)] However, the anti-inflammatory activity and anti-allergic activity of tannins have not yet been clarified.

Hyaluronidase is one of the mucopolysaccharide-splitting enzymes, and is related to the permeability of the vascular system[11,12)] and to inflammation.[13-15)] We have reported[16)] that anti-allergic agents such as disodium cromoglycate (DSCG) and tranilast which are known to inhibit histamine release from mast cells induced by antigen-IgE antibody reaction, are strong inhibitors of hyaluronidase, whereas compound 48/80, a histamine releaser, was found to activate hyaluronidase.

In the present study, we tried to investigate the correlation between the inhibitory effects of tannins on the activation of inactive hyaluronidase and on the degranulation from rat mesentery mast cells induced by compound 48/80.

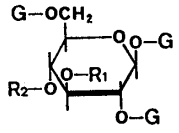
Results and Discussion

The inhibitory effects of tannins on the activation of inactive hyaluronidase and on the degranulation from rat mesentery mast cells induced by compound 48/80 were investigated and the results are shown in Figs. 1 and 2, respectively. It was found that all of the tested tannins inhibited the activation of inactive hyaluronidase and the degranulation from rat mesentery mast cells induced by compound 48/80 concentration-dependently. Furthermore,

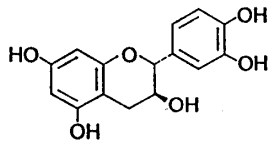
these inhibitory effects appeared to be well correlated. In both inhibitory effects, 1,2,3,4,6-penta-*O*-galloylglucose, 1,2,3,6-tetra-*O*-galloylglucose (hydrolyzable tannins) and 3,3′-di-*O*-galloylprodelphinidin B-2 (condensed tannin) which have some galloyl groups, showed about one hundred times stronger activity than the other tannins which do not have galloyl groups. The inhibitory effects seemed to increase with the number of galloyl groups of these tannins.

It is well known that hyaluronidase is one of the lysosomal enzymes[17] which is related to inflammation, and we have already reported[16] that various drugs which affect histamine release from mast cells modulated the activity of hyaluronidase. The degranulation and histamine release from mast cells are essential steps in the pathological mechanisms of type I allergy.[18,19]

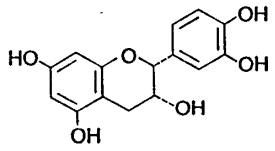
The above results suggest that tannins may possess anti-inflammatory activity and anti-allergic activity, and galloyl groups may be essential for the inhibitory effects not only on the



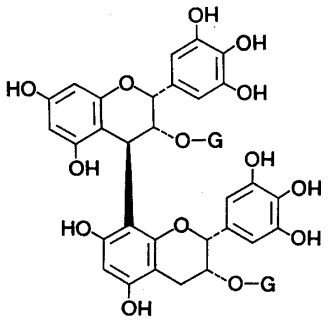
1,2,3,4,6-penta-*O*-galloylglucose: $R_1=R_2=G$, 1,2,3,6-tetra-*O*-galloylglucose: $R_1=G$, $R_2=H$, 1,2,6-tri-*O*-galloylglucose: $R_1=R_2=H$, G = galloyl.



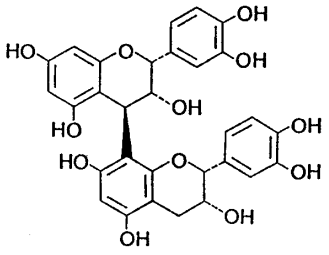
(+)-catechin



(−)-epicatechin



3,3′-di-*O*-galloylprodelphinidin B-2



procyanidin B-2

Chart 1. Chemical structures of tannins

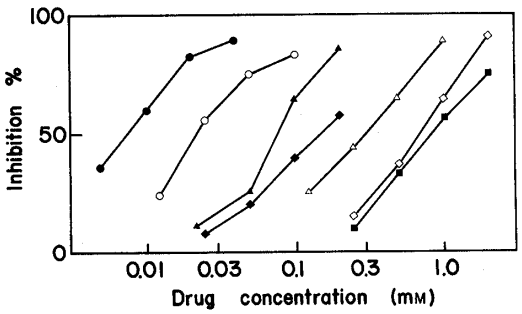


Fig. 1. Inhibitory Effects of Tannins on the Activation of Hyaluronidase

$CaCl_2$ (2.5 mM) was used as an activator of hyaluronidase.

Each point indicates the mean of 3 observations.

●, 1,2,3,4,6-penta-*O*-galloylglucose; ▲, 1,2,3,6-tetra-*O*-galloylglucose; ◆, 1,2,6-tri-*O*-galloylglucose; ○, 3,3′-di-*O*-galloylprodelphinidin B-2; △, procyanidin B-2; ◇, (−)-epicatechin; ■, (+)-catechin.

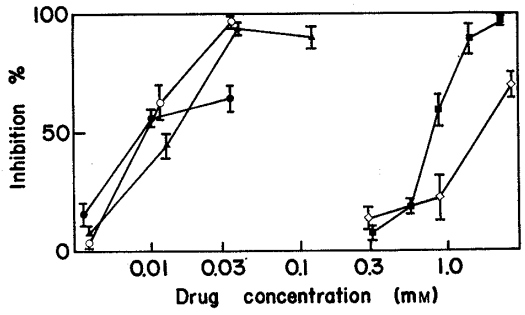


Fig. 2. Inhibitory Effects of Tannins on the Degranulation from Rat Mesentery Mast Cells Induced by Compound 48/80

Degranulation in the control rat mesentery mast cells was 80—90%. The concentration of compound 48/80 was 1.0 μg/ml.

Each point indicates the mean (±S.E.) of 4—6 observations.

●, 1,2,3,4,6-penta-*O*-galloylglucose; ▲, 1,2,3,6-tetra-*O*-galloylglucose; ○, 3,3′-di-*O*-galloylprodelphinidin B-2; ◇, (−)-epicatechin; ■, (+)-catechin.

activation of inactive hyaluronidase but also on the degranulation from rat mesentery mast cells induced by compound 48/80. Thus, tannins may be useful starting compounds for the development of new anti-inflammatory agents and anti-allergic agents.

Experimental

Materials——Hyaluronidase (from bovine testes) was purchased from Sigma Chemical Co., St. Louis; its specific activity was 500 NFunit/mg protein. Hyaluronic acid potassium salt was purchased from Wako Pure Chemical Co., Osaka, and compound 48/80 was purchased from Sigma Chemical Co. All of the tested tannins were isolated by the methods described in previous papers.[2)]

Assay of Hyaluronidase Activity——Hyaluronidase activity was determined by the Morgan-Elson method[20)] as modified by Davidson *et al.*[17)] after incubation of 340 NFunit/ml of hyaluronidase with 0.6 mg/ml hyaluronic acid potassium salt at 37 °C for 40 min in 0.1 M acetate buffer of pH 3.5. Calcium chloride (2.5 mM) was used as the activator of hyaluronidase.

Inhibitory Effects of Tannins on the Activation of Inactive Hyaluronidase——Inhibitory effects of tannins on the activation of inactive hyaluronidase were determined by the above method after incubation at 37 °C for 20 min in acetate buffer of pH 3.5 containing the activator and hyaluronidase which had been preincubated with the tannins at 37 °C for 20 min in the same buffer. Buffer solution was added in place of the buffer solution of tannins as a control. The percentage inhibition was calculated as follows:

$$\text{inhibition } (\%) = \frac{\text{control OD}_{585} - \text{sample OD}_{585}}{\text{control OD}_{585}} \times 100$$

Inhibitory Effects of Tannins on the Degranulation from Rat Mesentery Mast Cells Induced by Compound 48/80——The degranulation from rat mesentery mast cells was measured according to the modified Rothschild method.[21)] Male Wistar rats weighing 200—250 g were sacrified by means of a blow on the head and the mesenteries were excised and immersed in modified Lock's solution, which consists of 150 mM NaCl, 5.5 mM KCl, 2.1 mM $CaCl_2$, 0.7 mM $NaHCO_3$, 5.4 mM glucose, 1.6 mM Na_2HPO_4 and 1.2 mM $MgSO_4$. The mesenteries were cut into pieces, and incubated at 37 °C for 5 min in 4.0 ml of modified Lock's solution containing various concentrations of test sample. Modified Lock's solution (0.4 ml) was used in place of the same solution of test sample as a control. Then 1.0 ml of physiological solution of compound 48/80 (5.0 μg/ml) was added, and the system was incubated at 37 °C for 10 min. The pieces of mesentery were taken out, immersed in 10% formalin cooled in ice, and stirred with toluidine blue–acetic acid–formalin–water mixture (1 : 100 : 4 : 860, v/v) for 5 min. They were then washed with 50% ethanol, spread out on a slide glass and allowed to dry. Degranulated mast cells were counted under a microscope (× 200, Olympus Vanox, model AHB); 500 mast cells were counted in each sample. The percentages of degranulation and inhibition were calculated as follows:

$$\text{degranulation } (\%) = \frac{\text{degranulated mast cells (in 500)}}{500} \times 100$$

$$\text{inhibition } (\%) = 100 - \left(\frac{\text{degranulation (\%) of sample}}{\text{degranulation (\%) of control}} \times 100 \right)$$

References and Notes

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