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Anti-Allergic Action of Resveratrol and Related Hydroxystilbenes

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Abstract: **Resveratrol (1) and nine related hydroxystilbene compounds (2 ~ 9) isolated from plants were evaluated for the anti-allergic activities *in vitro*. Resveratrol (1) and rhapontigenin (5) demonstrated significant inhibitions upon the release of β-hexosaminidase from the cultured RBL-2H3 cells in a dose-dependent manner and the IC_{50} values were calculated as 14 and 15 μM, respectively.**

Resveratrol (**1**) is a representative of the hydroxystilbene compound class that occurs in many plants and is especially abundant in grapevine. It has received the special attention of many chemists and clinicians since it was known to have a cancer chemopreventive activity (1). Compound **1** also possesses a variety of biological activities such as, an anti-inflammatory effect due to the inhibition of the cyclooxygenase (1, 2), an anti-platelet activity (3), a protective action against lipid peroxidation (4), an estrogenic activity (5), and an inhibitory activity upon the monoamine oxidase (MAO-1) and DOPA oxidase (6, 7).

β-Hexosaminidase is stored in the secretory granules of mast cells, and is released along with histamine when mast cells are immunologically activated. It has been employed as a marker molecule for the degranulation process of mast cells and for estimating the anti-allergic activity *in vitro* (8, 9).

Naturally occurring hydroxystilbenes including **1** generally consist of two benzene rings connected through an olefin. The structural features of hydroxystilbenes (C_6–C_2–C_6) are similar to those of flavonoids (C_6–C_3–C_6) whose anti-allergic activities have been firmly established (10). This suggested that the hydroxystilbenes might also exert an anti-allergic activity *in vitro* as flavonoids do. Therefore, we have investigated the anti-allergic activities of **1** and several structurally related hydroxystilbenes (**2** ~ **9**) by estimating their inhibitory effects upon the release of β-hexosaminidase from the cultured mast cell (RBL-2H3 cells).

All examined hydroxystilbenes, except the glycoside compounds **7** – **10**, demonstrated significant inhibitions in a dose-dependent manner upon the release of β-hexosaminidase (Table **1** and Fig. **1**). Resveratrol (**1**) and rhapontigenin (**5**) were shown to possess relatively strong activities (IC_{50} values were calculated as 15 and 14 μM, respectively), and this indicated that the presence of two benzene rings with an appropriate distance as observed in the stilbenes or flavonoids might be essential to their common inhibitory effect on the release of β-hexosaminidase from the cultured mast cell (RBL-2H3 cells).

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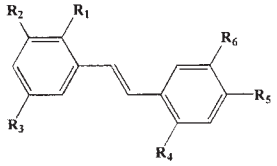
Table 1 Inhibitory effect of hydroxystilbenes upon the release of β-hexosaminidase.



	R_1	R_2	R_3	R_4	R_5	R_6	$IC_{50}(\mu M)^a$
1	-H	-OH	-OH	-H	-OH	-H	15.3±2.2[b]
2	-H	-OH	-OH	-H	$-OCH_3$	-H	24.5±3.1
3	-H	-OH	$-OCH_3$	-H	$-OCH_3$	-H	50.1±4.3
4	-H	$-OCH_3$	$-OCH_3$	-H	$-OCH_3$	-H	56.2±5.8
5	-H	-OH	-OH	-H	$-OCH_3$	-OH	14.2±2.3
6	-H	-OH	-OH	-OH	-OH	-H	32.3±2.8
7	-H	-OGlc	-OH	-H	-OH	-H	>100
8	-H	-OGlc	-OH	-H	$-OCH_3$	-H	>100
9	-H	-OGlc	-OH	-H	$-OCH_3$	-OH	>100
10	-OGlc	-OH	-OH	-H	-OH	-H	>100
Quercetin							3.4±2.0
Ketotifen							>100
DSSG							>100

[a] IC_{50} value of compound was defined as a concentration (μM) that caused 50 % inhibition on the release of β-hexosaminidase from cultured RBL-2H3 cells *in vitro*.

[b] Data are presented as mean ± S.E.M. of three distinct experiments.

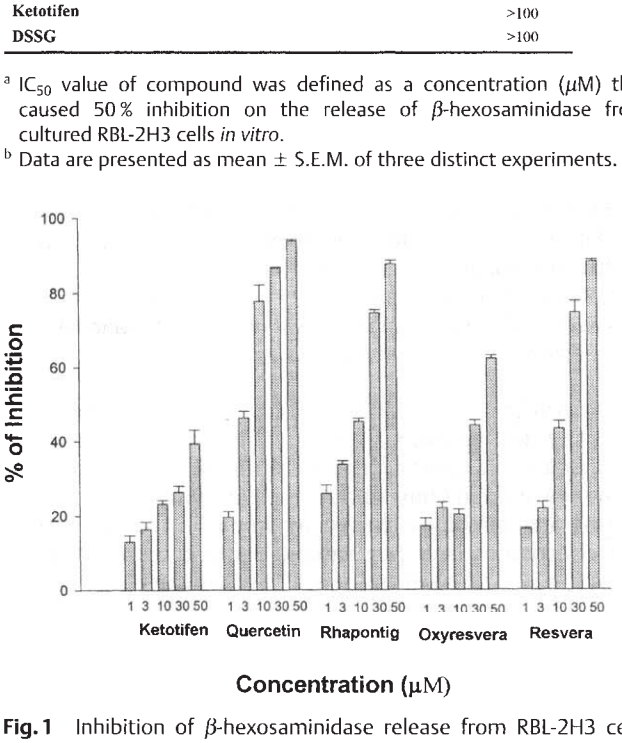


Fig. 1 Inhibition of β-hexosaminidase release from RBL-2H3 cells by hydroxystilbenes. The spontaneous release of β-hexosaminidase was measured to eliminate non-specific components of degranulation. Blank absorbance of test material itself was measured to eliminate the interferences caused by the color of the test material itself. Thus, the inhibition % of the release of β-hexosaminidase by test material was calculated by following equation. Each test was conducted in triplicate and the mean inhibition % and S.E.M. was calculated. Resvera is the abbreviation for resveratrol, oxyresvera is for oxyresveratrol, and rhapontig is for rhapontigenin.
% of inhibition = (Treated − Blank − Spontaneous)/(Control − Blank − Spontaneous)
Control: normal allergen-IgE response was evoked, test material was not added.
Treated: normal allergen-IgE response was evoked, test material was added.
Blank: only test material and substrate were added into ELISA plate.
Spontaneous: allergen-IgE response was not evoked, test material was not added.

The activities of resveratrol and rhapontigenin were much stronger than those of DSSG or ketotifen (IC_{50} values of two compounds were around 100 μM). This is understandable because the mechanism of anti-allergic actions of ketotifen involves the inhibition of histamine release (10) but the main one is anti-histamine action (11), and that of DSSG remains unclear. Meanwhile, the activities of resveratrol and rhapontigenin were comparable with that of quercetin (IC_{50} value was ca 3 μM), a flavonoid with prominent anti-allergic activity (12).

Interestingly, as the three hydroxy groups of resveratrol (**1**) were replaced stepwise with methoxy groups to give **2**~**4**, the anti-allergic effect decreased in proportion to the number of methoxy groups (IC_{50} value increased in the order of **1** < **2** < **3** < **4**). Thus, it appears that the hydroxy substituents on the benzene rings of the hydroxystilbenes might be critical for the anti-allergic activity. However, none of the glycosides **7**–**10** exhibited significant anti-allergic activities below 100 μM. Recently, it was reported that some stilbenes such as **5** and **9** inhibited concanavalin A induced histamine release from human basophils (13). These results are well in accord with our observations.

Materials and Methods

Hydroxystilbenes: All hydroxystilbenes tested were isolated from herbal extracts or obtained by chemical modifications (6). Resveratrol (3,4′,5-trihydroxystilbene; **1**) was isolated from the roots of *Veratrum album* var. *grandiflorum*. The 3,5-dihydroxy-4′-methoxystilbene (**2**), rhapontigenin (**5**), 3,5-dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside (**8**), rhapontin (**9**) were from *Rheum undulatum*. Piceid (resveratrol-3-*O*-glucoside; **7**) was isolated from *Polygonum cuspidatum*, oxyresveratrol (**6**) from *Morus alba* and 2,3,4′,5-tetrahydroxystilbene-2-*O*-β-D-glucoside (**10**) from *Polygonum multiflorum*. Trimethylresveratrol (**4**) was prepared by methylation of resveratrol (**1**) with diazomethane; 3,4′-dimethoxy-5-hydroxystilbene (**3**) was obtained by methylation of piceid (**7**) followed by the acid hydrolysis. All compounds were further purified by HPLC before experiments and the purity was higher than 95 %.

Details of isolation procedure and copies of the original spectra are obtainable from the author of correspondence.

Inhibition of β-hexosaminidase release from RBL-2H3 cells: The release of β-hexosaminidase from RBL-2H3 cells was evaluated according to Cheong et al. (9).

Acknowledgements

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The Effects of Tetramethylpyrazine on the Incidence of Arrhythmias and the Release of PGI_2 and TXA_2 in the Ischemic Rat Heart

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Abstract: **Pretreatment with tetramethylpyrazine (TMP, 12 mg/kg/day), a drug originally derived from the rhizomes of *Ligusticum wallichii*, significantly reduced the incidence of ischemia-induced ventricular tachycardia (VT) and fibrillation (VF) from 100 % and 50 % of control hearts to 41 % ($p < 0.05$) and 0 % ($p < 0.05$), respectively, in the ischemic rat heart. TMP also diminished the incidence of reperfusion-induced VT and VF from 100 % and 100 % of control hearts to 33 % ($p < 0.05$) and 41 % ($p < 0.05$), respectively. Pretreatment with TMP produced a slight, but significant increase of 6-keto-$PGF_{1\alpha}$ and a decrease of TXB_2 production during aerobic perfusion. Ischemia and reperfusion markedly increased the release of 6-keto-$PGF_{1\alpha}$ and TXB_2. Pretreatment with TMP significantly enhanced the release of 6-keto-$PGF_{1\alpha}$ and diminished TXB_2 outflow following left coronary artery occlusion and reperfusion.**

Tetramethylpyrazine (TMP) is a biologically active component originally derived from a popular Chinese herbal medicine, the rhizomes of *Ligusticum wallichii* (**1**, Fig. **1**). TMP has been rountinely used in China to treat patients with ischemic heart disease and cerebral ischemic syndrome (2) due to its biological activities including vasodilation and antiplatelet aggregation (1, 3). However, the real mechanism of the beneficial effects of TMP is still unresolved. We have previously shown that TMP could protect the rat heart against myocardial dysfunction and influence the release of prostacyclin (PGI_2) and thromboxane (TXA_2) following global ischemia (4). More recently, our data indicated that pretreatment with TMP for 7 days increased the release of PGI_2 and decreased TXA_2 in rat heart under both normoxic and hypoxic conditions (5, 6). To our knowledge, there is little information concerning the effect of TMP on the incidence of ventricular arrhythmias following ischemia and reperfusion. It is also interesting to know whether pretreatment with TMP may affect PGI_2 and TXA_2 release following regional ischemia and reperfusion in the rat heart. The aims of the present study were, therefore, to evaluate the effects of pretreatment with TMP for 7 days on the incidences of ischemia and reperfusion induced arrhythmias and the changes in PGI_2 and TXA_2 production by measuring their stable metabolites, 6-keto-$PGF_{1\alpha}$ and TXB_2 in ischemic rat heart perfused with blood free solution.

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