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Inhibition of Human Tumor Growth by 2′-Hydroxy- and 2′-Benzoyloxycinnamaldehydes

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Abstract: **2′-Hydroxycinnamaldehyde (HCA) was isolated from *Cinnamomum cassia* Blume (Lauraceae) and 2′-benzoyloxycinnamaldehyde (BCA) was prepared by the reaction of HCA and benzoyl chloride. HCA and BCA strongly inhibited *in vitro* growth of 29 kinds of human cancer cells and *in vivo* growth of SW-620 human tumor xenograft without the loss of body weight in nude mice. HCA prevented adherence of SW-620 cells to the culture surface but did not inhibit oncogenic K-Ras processing, implying its antitumor mechanisms at the cellular level.**

The extract of *Cinnamomum cassia* Blume (Lauraceae) has been used as a therapeutic agent for various diseases including hypertension and indigestion in traditional Asian medicine (1, 2). We reported in our previous paper that 2′-hydroxycinnamaldehyde (HCA, Fig. **1**) was first purified from the stem bark of *Cinnamomum cassia* Blume. HCA inhibited farnesyl transferase (FTase), which was determined by *in vitro* scintillation proximity assay (SPA) (1). Also, the antiangiogenic activity of HCA was revealed by chick embryo chorioallantonic membrane (CAM) assay (2). Antitumor activity of various cinnamaldehyde derivatives was investigated against leukemia L1210, but they did not show significant antitumor activity (3). Here, we describe *in vitro* and *in vivo* antitumor activity of HCA and its derivative, 2′-benzoylxycinnamaldehyde (BCA, Fig. **1**).

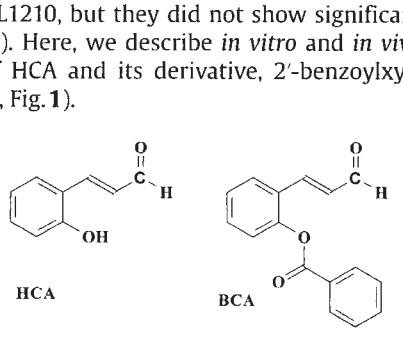

Fig. 1 Structures of HCA and BCA.

HCA and BCA showed strong *in vitro* growth inhibition of 29 kinds of human cancer cells and the panel of human tumor cell lines tested is summarized in Table **1**. The average concentration of HCA and BCA for *in vitro* growth inhibition by 50% (GI_{50}) was calculated to be 2.64 and 3.86 μg/ml, respectively. Some cell lines, such as A549, NCI-H522, Caki-1, and Colo-205, were relatively resistant, exhibiting GI_{50}s in the range of 6 to 12 μg/ml.

Next, tumor growth inhibition activity of these compounds was evaluated in a human tumor xenograft model of nude mice. HCA and BCA strongly inhibit the growth of tumors (Fig. **2A**, **B**) and also significantly reduced the weight of tumors excised on the final day (Fig. **2C**). These results provide strong evidence that these compounds have the potential for use as anticancer agents. A promising aspect of HCA and BCA was their apparent lack of obvious toxicity in tumor-bearing nude mice (Fig. **3**). The loss of body weight was not observed in animals administered with HCA and BCA at effective concentrations showing tumor growth inhibition. However, treatment with adriamycin resulted in the reduction of body weight, although showing strong antitumor activity. The relative lack of the toxicity of HCA and BCA contrasts with the toxic nature of currently available chemotherapeutic agents.

Table 1 Growth inhibition by HCA and BCA against 29 kinds of human cancer cell lines (GI_{50}, μg/ml).

Organ	Cell Line	HCA	BCA	Adriamycin
Lung	A549	9.39	11.98	0.44
	NCI-H226	3.87	4.48	0.36
	HCI-H23	0.87	1.24	0.17
	NCI-H522	6.83	10.88	0.36
Renal	UO-31	1.15	3.95	0.20
	ACHN	1.13	1.78	0.31
	Caki-1	6.05	6.75	0.49
Colon	Colo205	8.26	7.70	0.19
	HCT15	3.03	3.75	0.84
	HT29	3.28	4.96	0.34
	HCT116	1.31	1.96	0.17
	KM12	2.92	2.41	0.30
	SW620	1.27	1.22	0.14
CNS	SF539	1.09	3.31	0.074
	SNB19	1.76	3.48	0.14
	SNB75	2.19	2.61	0.16
Melanoma	UACC62	0.91	1.80	0.11
	LOX-IMVI	1.19	1.86	0.25
	M14	1.89	1.70	0.059
	SK-MEL-2	2.34	3.56	0.41
Breast	MCF7	1.74	2.90	0.15
	MCF7/ADR	1.61	2.78	0.97
	MDA-MB-231	0.998	2.21	0.27
Leukemia	RPMI8226	1.33	1.87	0.63
	K562	2.96	2.96	0.42
	MOLT-4F	1.83	1.57	0.14
Ovary	SK-OV-3	1.67	8.92	0.13
	OVCAR-4	2.00	4.84	0.48
Prostate	PC-3	1.58	2.37	0.47
	Average	2.636	3.855	0.32

Growth inhibition by HCA and BCA against 29 kinds of human cancer cell lines was evaluated by sulforhodamine B (SRB) assay (4). Cancer cells were inoculated to 96 well plates at a concentration of 10,000 cells/well and cultured in RPM11640 containing 5% serum for 24 h. After addition of compounds, the cancer cells were further incubated for 48 h. After incubation, 50 μl of 50% trichloroacetic acid was added. After fixation for 1 h at 4 °C, plates were washed with tap water several times and air-dried. Fixed cells were stained for 30 min with SRB (0.4% in 1% acetic acid). After staining, the unbound dye was washed out with 1% acetic acid and air-dried. To each well of the plates, 100 μl of Tris base (10 mM, pH 10.5) was added. Optical density was measured with a microplate reader (Molecular Devices, Model Emax) at 540 nm.

The antitumor mechanisms of HCA were examined *in vitro* by using anchorage-dependent SW-620 cells. The treatment with HCA made anchorage-dependent SW-620 cells float on the culture medium in a dose-dependent manner, resulting in the increase of detached cells and the decrease of attached cells (Table **2**). However, the viability of each population was not affected or only slightly affected up to 5 μg/ml, which was higher than GI_{50}. These results suggested that HCA primarily prevented the adherence of SW-620 cells without direct cytotoxicity, followed by growth cessation of HCA-treated cells. This phenomenon was different from the growth inhibition activity of adriamycin in that cytotoxicity of adriamycin resulted in a rapid breakdown of affected cells. Detached, viable cells were rarely observed in the adriamycin-treated culture.

In a previous paper, we reported that HCA inhibited FTase showing at 22 μg/ml 50% inhibition concentration (IC_{50}) in the *in vitro* SPA enzyme assay and this prompted us to investigate the FTase inhibition activity of HCA. We used SW-620 cells to investigate whether HCA inhibited oncogenic K-Ras processing at the cellular level. However, their Ras

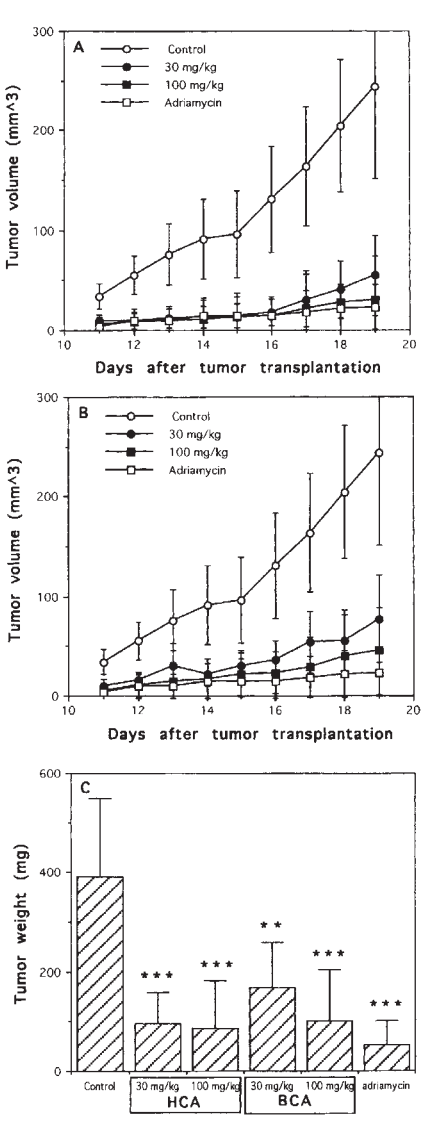

Fig. 2 Effects of HCA and BCA on tumor growth.

proteins were fully processed by FTase and unprocessed Ras proteins were not observed, although HCA inhibited the growth of SW-620 cancer cells (unpublished data). This finding indicated that the antitumor activity of HCA was not related with the inhibition of K-Ras processing.

The antitumor activity of HCA and BCA might be mediated by various independent mechanisms. At the cellular level, the present compounds might have a cytostatic nature, which might be induced by the disturbance of cell adherence. The *in vivo* antitumor activity seemed to be mediated by the antiangiogenic potential as well. HCA inhibited angiogenesis showing a higher potency than well-known angiogenesis inhibitors such as AGM-1470 and genistein (2). However, the effect of HCA on FTase might not be involved in actual growth inhibition of human tumors at the cellular level, although FTase inhibition by HCA was proposed in our previous *in vitro* enzyme assay. In conclusion, the growth inhibitory and antitumor action of HCA and BCA in cell culture and animal studies supported their promise as antitumor drugs. The relative lack of toxicity in animal studies was a pleasant aspect of HCA and BCA. Further studies will be needed to identify the exact mechanisms and the possibility of HCA and BCA as clinically efficient antitumor drugs.

Table 2 Viability changes of SW-620 cells by the *in vitro* treatment with HCA.

Concentrations	Adherent cells	Detached cells
Control	72[a] (88.2)[b]	1 (76.7)
1 μg/ml	36 (84.6)	3 (78.5)
3 μg/ml	21 (81.1)	5 (76.4)
5 μg/ml	11 (76.3)	15 (66.6)
7 μg/ml	6 (65.5)	25 (45.4)

SW-620 cells were seeded into 24 well plates at a concentration of 20 × 10^4 cells/ml and cultured in RPM11640 containing 10 % fetal calf serum for 24 h. After treatment of HCA (1 ~ 7 μg/ml), cancer cells were further incubated for 24 h. Adherent cells were harvested by using 0.05 % trypsin/0.53 mM EDTA and detached cells were harvested by pipetting.
[a] Total cell number (× 10^4 cells/ml) was enumerated by hemacytometer.
[b] Viability (%) was determined by the flow cytometric analysis using 5 μg/ml propidium iodide.

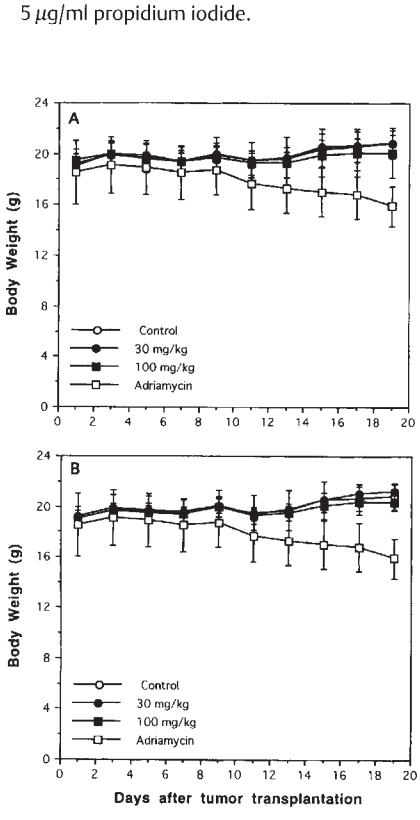

Fig. 3 Toxicity of HCA and BCA.

Materials and Methods

HCA was isolated from cinnamon bark by the methods mentioned previously (1). BCA was prepared by the reaction of HCA with benzoyl chloride in the presence of triethylamine. Triethylamine (1 ml) was added to the HCA solution (50 g/50 ml of CH_2Cl_2) under nitrogen. After cooling, the mixture was mixed with benzoyl chloride (0.1 ml) and stirred for 30 min at 0 °C. After mixing with sodium hydroxide (50 ml, pH 9) and 50 ml CH_2Cl_2, the organic layer was collected, dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was subjected to flash chromatography (E. Merk, 50 g silica gel 60, 230 – 240 mesh) with a gradient of hexane-ethyl acetate (9 : 1 to 7 : 3 v/v). The yield of BCA was 42 mg in powder form. The structure of BCA was determined by various analytical methods such as mass spectrometry, IR, and NMR.

In vitro growth inhibition by HCA and BCA against 29 kinds of human cancer cell lines was evaluated by the sulforhodamine B (SRB) assay (4). For the evaluation of *in vivo* antitumor activity of HCA and BCA, SW-620 human colon adenocarcinoma cells (1 × 10^7 cells/ml) were implanted subcutaneously into the right flank of nude mice on day 0. Tumor volumes were estimated by the formula length (mm) × width (mm) × height (mm)/2 (5). On day 19, the mice were sacrificed and the tumors were weighed. Compounds were dissolved in 0.5 % tween80 and were intraperitoneously administered at concentrations of 30 and 100 mg/kg from day one to day 18. Adriamycin was used as a reference compound and its dosage was changed to show clear antitumor activity such as the following; 1 mg/kg from day 1 to day 11, 2 mg/kg from day 12 to day 14, and 3 mg/kg from day 15 to day 18. The amount of dosage was 0.2 ml per 20 g body weight of animals. To determine toxicity of the compounds, the body weight of tumor-bearing animals was measured. Inhibition on the K-Ras processing by HCA was determined by using adherent SW-620 cells (6).

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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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