Cytotoxicity of Urushiols Isolated from Sap of Korean Lacquer Tree (*Rhus vernicifera* Stokes)

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(Received November 29, 1999)

Cytotoxicities of four urushiols, congeners isolated from the sap of Korean lacquer tree (*Rhus vernicifera* Stokes), to 29 human cancer cell lines originated from 9 organs were evaluated. Their values of 50% growth inhibition were below 4 μg/ml, and showed cell line specific cytotoxicity. The present result is the first report on the cytotoxicity of urushiols suggesting that they would have an anticancer activity to human cancer cells.

Key words: Urushiols, Cytotoxicity, Human Caner Cells

INTRODUCTION

The plant family *Anacardiaceae* contains skin sensitizing agents in their resins. These allergenic agents, commonly referred to as urushiols, are mixtures of olefinic catechols with a n-C_{15} or n-C_{17} alkyl side chain (Watson *et al.*, 1981). Urushiol processed by $CD8^+$ T cells, but not by $CD4^+$ T cells, could be able to evoke contact dermatitis (Kalish and Johnson, 1990). Although urushiol has been extensively studied on its allergic contact dermatitis, the cytotoxic activity was not studied until now (Fraginals *et al.*, 1991; Kalish *et al.*, 1994). The main purpose of this study was to determine cytotoxic activities of urushiols isolated from the sap of Korean lacquer tree (*Rhus vernicifera* Stokes).

MATERIALS AND METHODS

Isolation of urushiol

The sap of lacquer tree was obtained from Wonju, Kwangwon Province, Korea, which is a main production area of Korean lacquer tree, in October 1995. The sap (37 ml) of lacquer tree was diluted to a volume of 1 L by the addition of distilled water and extracted with 1 L of *n*-hexane twice. The hexane extract was concentrated under reduced pressure to afford a brownish oil (24.4 g). It was purified by a silica gel column chromatography (Merck 7734) and eluted with 20% acetone/hexane. It was further purified by the same methods (Merck 9385), followed purified by ODS gel column chromatography (YMC GEL ODS-A) with gradient of methanol in water to give four urushiols (compound 1, 1,138 mg; compound 2, 159 mg; compound 3, 13 mg; compound 4, 19 mg). The full purification steps were described in the previous paper (Kim *et al.*, 1997).

Cytotoxic sulforhodamine B (SRB) assay

Twenty-nine kinds of human cancer cell lines were used and cultured with RPMI 1640 containing 10% fetal calf serum (FCS). For SRB assay, cells were cultured in RPMI 1640 containing 5% FCS (R5). SRB assay was performed by previous method (Kim et *al.*, 1996). Cell suspension (3~40,000 cells/ml) was made in culture medium and inoculated to each well of 96-well microtiter plate. One day after plating, time zero control plate was made, compounds (1~4) were directly treated, and cells were incubated for further 48 hrs in a CO_2 incubator. Cells were fixed with 50 μl of 50% trichloroacetic acid solution for 1 h at 4°C and plates were washed 5 times with tap water and air-dried. 100 μl of SRB solution (0.4% in 1% acetic acid) was added and staining was done at room temperature for 30 min. Residual dye was washed out with 1% acetic acid and air-dried. To each well, Tris solution (10 mM, pH 10.5) was added. Optical density (OD) was measured with microtiter plate reader at 540 nm. Growth inhibition was calculated according to the previous method. Briefly, OD of treated well was subtracted OD at time-zero (Tz) plate and divided by calculated value of untreated

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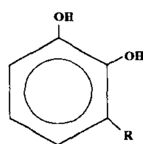

Compound 1	R = $(CH_2)_7CH{=}CHCH_2CH{=}CHCH_2CH{=}CH_2$	(M.W. = 314)
Compound 2	R = $(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_2CH_3$	(M.W. = 316)
Compound 3	R = $(CH_2)_7CH{=}CH(CH_2)_5CH_3$	(M.W. = 318)
Compound 4	R = $(CH_2)_{14}CH_3$	(M.W. = 320)

Fig. 1. Structures of four urushiol derivatives isolated from the sap of Korean lacqure tree (*Rhus vernicifera*).

control. Growth inhibition of 50% (GI_{50}) was calculated by Probit method (Wu *et al.*, 1992).

RESULTS AND DISCUSSION

The sap of Korean lacquer tree was partitioned with *n*-hexane and water. The hexane-soluble portion was subjected to silica and then ODS gel column chromatography. The cytotoxic components of this portion were identified as four olefinic catechols by the comparison of their ^{1}H-NMR, EI-MS, IR and UV with the data reported

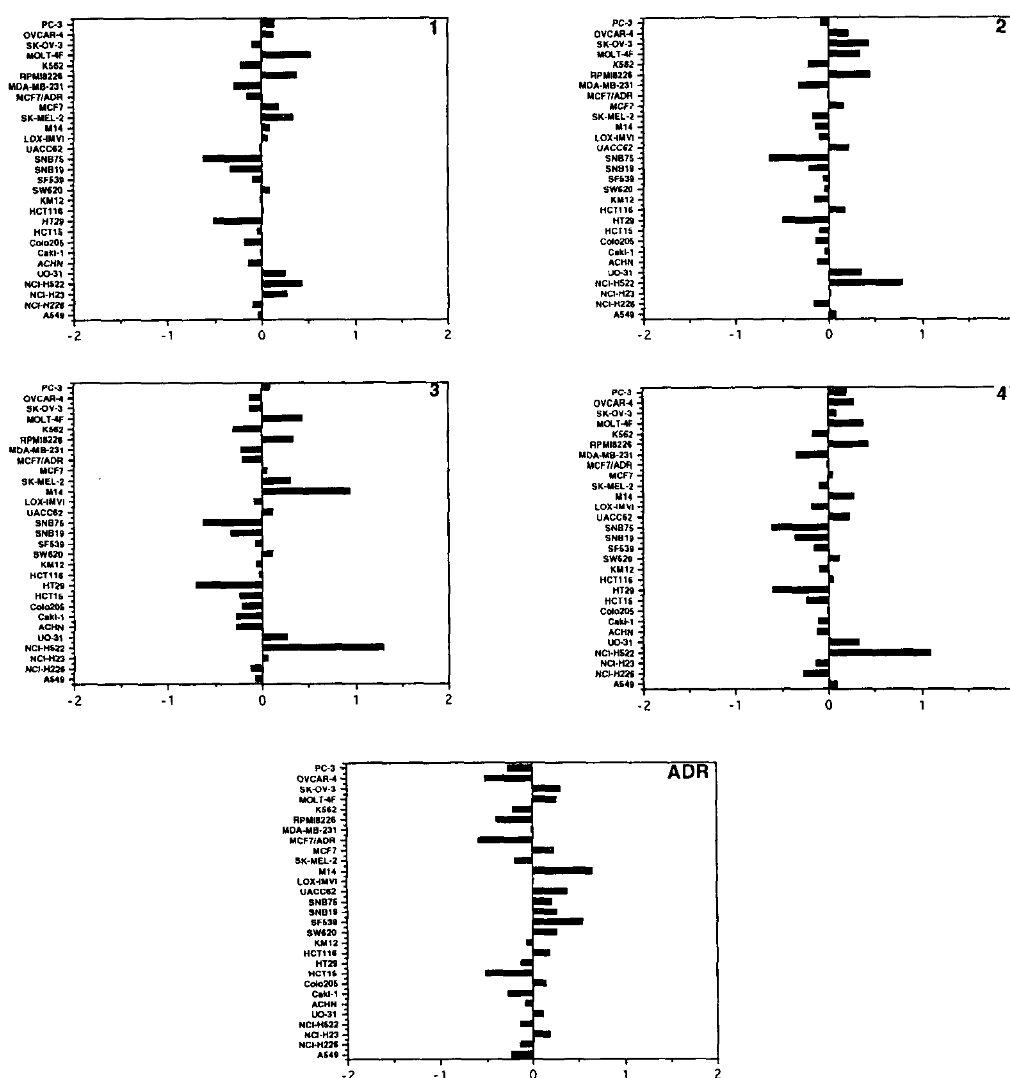

Fig. 2. Mean graph from the human cancer cell line panel. Centered on an axis indicates that the compound yielded average GI_{50} values. For each agent the difference between the $\log_{10}$ of each cell line and the mean GI_{50} log for that agent is determined, to yield negative values for cell lines more resistant than average (*bars projecting to the left*) and positive values for cell lines more sensitive than average (*bars projecting to the right*). The cell lines used in the present studies: prostate lines PC-3; ovarian carcinoma lines OVCAR-4 and SK-OV-3; leukemia lines MOLT-4F, K562, and RPMI8226; breast lines MDA-MB-235, MCF7/ADR, and MCF7; melanoma lines SK-MEL-2, M14, LOX-IMVI, and UACC62; central nervous system cancer lines SNB-75, SNB-19, and SF539; colon carcinoma lines SW620, KM12, HCT-116, HCT-15, and COLO205; renal carcinoma lines CAKI-1, ACHN, and UO-31; and lung carcinoma lines NCI-H522, NCI-H23, NCI-H226, and A549.

Table I. Cytotoxcity of four compounds (50% growth inhibition(GI_{50}) concentration)

Cell Line	Origin	1	2	3	4	ADR
A549	Lung	2.22	1.96	2.34	2.01	0.44
NCI-H226		2.55	3.32	2.68	4.63	0.36
NCI-H23		1.13	2.21	1.79	3.31	0.17
NCI-H522		0.77	0.38	< 0.10	0.20	0.36
UO-31	Renal	1.17	1.04	1.11	1.16	0.20
ACHN		2.80	3.08	3.00	3.23	0.31
Caki-1		2.08	2.53	3.77	3.17	0.49
Colo205	Colon	3.10	3.16	3.19	2.54	0.19
HCT15		2.25	2.88	3.43	4.18	0.84
HT29		6.54	7.13	> 10.0	> 10.0	0.34
HCT116		2.01	1.56	2.17	2.23	0.17
KM12		2.10	3.24	2.27	3.07	0.30
SW620		1.70	2.50	1.56	1.87	0.14
SF539	CNS	2.53	2.62	2.32	3.45	0.074
SNB19		4.40	3.71	4.28	5.57	0.14
SNB75		8.61	> 10.0	8.24	> 10.0	0.16
UACC62	Melanoma	2.10	1.43	1.57	1.48	0.11
LOX-IMVI		1.79	2.90	2.45	3.69	0.25
M14		1.71	3.14	0.23	1.31	0.059
SK-MEL-2		0.96	3.34	1.01	3.04	0.41
MCF7	Breast	1.35	1.60	1.78	2.20	0.15
MCF7/ADR		2.88	2.40	3.22	2.51	0.97
MDA-MB-231		3.98	4.80	3.38	5.39	0.27
RPMI8226	Leukemia	0.88	0.83	0.95	0.90	0.63
K562		3.42	3.82	4.09	3.62	0.42
MOLT-4F		0.61	0.89	0.74	1.04	0.14
SK-OV-3	Ovary	2.56	1.08	2.69	2.00	0.13
OVCAR-4		1.52	0.85	2.69	1.30	0.48
PC-3	Prostate	1.50	1.42	1.68	1.54	0.47
	Mean	2.46	2.75	2.71	3.13	0.32

in the literatures (Fig. 1) (Kim *et al.*, 1997). The four components occupied much more than 25% of the sap. The major component was 1 (over 21% of the sap), followed by 2 (over 3% of the sap).

The cytotoxic effect of urushiol compounds was evaluated on 29 kinds of human cancer cell lines (Table I). The values of 50% growth inhibition (GI_{50}) were mostly below 4 μg/ml for pure compounds to be considered as significantly active (Ali *et al.*, 1997). However, some cell lines such as HT29 and SNB75 showed relatively high GI_{50} values. Others such as NCI-H522, RPMI8226, and MOLT- 4F showed relatively low GI_{50} values. The mean of four urushiolic compounds to various cell lines was similar, indicating that they were similar in cytotoxic potential. Adriamycin was highly cytotoxic compared to urushiol.

Fig. 2 shows the patterns of differential cytotoxicity of four urushiols. Like the result of Table I, HT-29 and SNB75 were less sensitive and NCI-H522, RPMI8226, and MOLT-4F were more sensitive than other cell lines.

In the present study, the cytotoxic acitivty of urushiols was firstly evaluated. Urushiols showed cell line specific cytotoxicity. Although cytotoxic potential of urushiols is weaker than that of adriamycin, they are worthy of further studying in a variety of *in vivo* tumor models. Also, they still show promise as an immunochemotherapeutic drug owing to theirs bifunctional activities on cancer. Immunostimulating activity on T cells and direct cytotoxic activity of urushiols might help inhibit tumor growth in a synergistic manner.

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