Antiviral Research, 18 (1992) 91–103

AVR 00550

Inhibition of human immunodeficiency viral replication by tannins and related compounds

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Received 26 August 1991; accepted 13 November 1991)

Summary

Among 87 chemically defined tannins and related compounds, several hydrolyzable tannins, but not condensed tannins or other lower molecular weight polyphenols, significantly inhibited both the cytopathic effect of human immunodeficiency virus (HIV) and the expression of HIV antigen in human lymphotropic virus type I (HTLV-1)-positive MT-4 cells. The 50% effective concentrations (2.0–4.8 μg/ml) of the active compounds were 13- to 15-fold lower than their 50% cytotoxic concentrations. Their anti-HIV activity was demonstrated to be mediated, at least in part, by inhibition of HIV adsorption to the cells.

Tannin; HIV; Virus adsorption

Introduction

Tannin-related compounds have been classified into two large groups: hydrolyzable and condensed tannins (Okuda et al., 1991). Hydrolyzable tannins have structures in which a polyalcohol (mainly glucose) is esterified with a polyphenolic carboxylic acid, such as a galloyl, hexahydroxydiphenoyl

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(HHDP) (a dimer of the galloyl group), valoneoyl (a trimer of the galloyl group) or dehydrohexahydroxydiphenoyl group (an oxidized metabolite of the HHDP group). Condensed tannins are composed of flavan units, mostly (+)-catechin, (−)-epicatechin, or their analogs, condensed with each other via carbon-carbon bonds.

Several tannin-related compounds, isolated from various Chinese drugs, have been reported to exert multiple biological effects such as antibacterial (Toda et al., 1990), antiviral (Fukuchi et al., 1989; Hatano et al., 1988), antihemolytic (Ikigai et al., 1990), antitumor (Miyamoto et al., 1987) and antitumor promoting (Yoshizawa et al., 1987) activity, and inhibitory activity against enzymes such as reverse transcriptase (Kakiuchi et al., 1985; Nishizawa et al., 1989), DNA polymerases (Parker et al., 1989) and poly(ADP-ribose) glycohydrolase (Tanuma et al., 1989). However, there is little information about the effect of tannins on human immunodeficiency virus (HIV) replication (Asanaka et al., 1987; Nonaka et al., 1990). Therefore, we investigated a total of 87 chemically defined tannins and related compounds for their ability to inhibit HIV replication. We monitored the cytopathic effect of HIV infection, the expression of HIV antigen, and the binding of HIV to the target cells in the presence of tannins.

Materials and Methods

Materials

The following tannins and related compounds were isolated from the plants indicated in parentheses and their structural information is available from our previous papers (Hatano et al., 1990; Okuda et al., 1990, 1991; Sakagami et al., 1990): 2,3-digalloylglucose, 1,2,6-trigalloylglucose, 1,2,3,6-tetragalloylglucose, tellimagrandins (I, II), isoterchebin, cornusiins (A, C) (*Cornus officinalis* Sieb. et Zucc.); penta-*O*-galloyl-β-D-glucose (produced from tannic acid (Dainippon Pharm. Co. Ltd., Osaka, Japan)); ECG ((−)-epicatechin 3-*O*-gallate), EGC ((−)-epigallocatechin), EGCG ((−)-epigallocatechin 3-*O*-gallate) (*Thea sinensis* L.); ECG-dimer, ECG-trimer, ECG-tetramer (*Saxifraga stolonifera* Meerb.); gemins (A, D) (*Geum japonicum* Thunb.); corilagin (produced from geraniin); 1,3-digalloyl-4,6-HHDP-glucose, hirtellins (A–E), remurins (A, B) (*Reaumuria hirtella* Jaub. et Sp.); isohirtellin C (produced from hirtellin C); tamarixinin A (*Tamarix pakistanica* Qaiser); casuarictin (*Casuarina stricta* Ait); geraniin, dehydrogeraniin (*Geranium thunbergii* Sieb. et Zucc.); carpinusin, antidesmin A (*Antidesma pentandrum* Merr. var. *barbatum* Merr.); chebulinic acid, chebulagic acid (*Terminalia chebula* Retz.); coriariins (A, B, D, E) (*Coriaria japonica* A. Gray.); nonacosa-*O*-methylcoriariin A (produced from coriariin A); schimawalin B, isoschimawalin A (*Schima wallichii* Korth.); camelliins (A, B), camelliatannin A (*Camellia japonica* L.); agrimoniin (*Agrimonia pilosa* Ledeb. var. *japonica* Nakai); isorugosins (D, E) (*Liqui-*

dambar formosana Hance); rugosins (D, E) (*Rosa rugosa* Thunb.); desgalloyl-rugosin F (*Corylus heterophylla* Fisch.); woodfordins (A–D, F, H, I), oenothein A (*Woodfordia fruticosa* Kurz); nobotanins (A–C, F) (*Tibouchina semidecandra* Cogn.); nobotanin K (*Heterocentron roseum* A. Br. et Bouche); euphorbins (A–E) (*Euphorbia hirta* L.); euphorbin F (*Euphorbia tirucalli* L.); euphorbins (G, H) (*Euphorbia prostrata* Ait.); trapanins (A, B) (*Trapa japonica* Flerov.); (+)-catechin, (−)-epicatechin, ellagic acid (Sigma), gallic acid (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan); methyl gallate, ethyl gallate, n-propyl gallate, n-butyl gallate, lauryl gallate, stearyl gallate (Fuji Chemical Ind., Wakayama, Japan); rosmarinic acid (*Perilla frutescens* Britton var. *crispa* Decne.); rabdosiin (*Rabdosia japonica* Hara); taxifolin apioside (*Rosa davurica* Pall.).The following reagents were obtained from the indicated companies: RPMI 1640 medium (Gibco, Grand Island, NY); fetal calf serum (FCS) (Whiptaker Bioproduct Inc., MD); 3-(4,5-dimethylthiazol-2-yl) 2,5-dephenyltetrazolium bromide (MTT) (Wako Pure Chemical Co., Ltd., Osaka, Japan), fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (Cappel Organon Teknika Co., West Chester, PA).

Cell lines

Human T lymphotropic virus type I (HTLV-1-)-positive T cell line, MT-4, was subcultured twice a week at a concentration of 3×10^5/ml in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, penicillin (100 IU/ml) and streptomycin (100 μg/ml).

Virus

A strain of HIV-1, HTLV-IIIB, was used in the anti-HIV assay. The virus was prepared from the culture supernatant of MOLT-4/HTLV-IIIB cells which were persistently infected with HTLV-IIIB. HIV stocks were titrated in MT-4 cells as determined by 50% tissue culture infectious doses ($TCID_{50}$) and plaque forming units (Harada et al., 1985) and stored at −80°C until used.

Assay for HIV-induced cytopathic effect

MT-4 cells were infected with HTLV-IIIB at a multiplicity of infection (MOI) of 0.01. HIV-infected or mock-infected MT-4 cells (1.5×10^5/ml, 200 μl) were placed into 96-well microtiter plates (Falcon 3071, Becton Dickinson, NJ) and incubated in the presence of varying concentrations of the test compounds. After incubation for 5 days at 37°C in a CO_2 incubator, cell viability was quantified by a colorimetric assay monitoring the ability of viable cells to reduce MTT to a blue formazan product. The absorbances were read in a microcomputer-controlled photometer (Titertek Multiskan®; Labsystem Oy, Helsinki, Finland) at two wavelengths (540 and 690 nm). The absorbance measured at 690 nm was automatically subtracted from the absorbance at 540

nm, so as to eliminate the effects of non-specific absorption. All data represent the mean values of triplicate measurements. The standard deviations of the results were usually below 5%. From the calibration curve, 50% cytotoxic concentration (CC_{50}), 50% effective concentration (EC_{50}) and selectivity indexes (SI) ($SI = CC_{50}/EC_{50}$) were calculated (Pauwels et al., 1988).

Immunofluorescence staining

HIV-infected cells were washed with phosphate buffered saline (PBS; pH 7.2) and reacted, first, with human polyclonal anti-HIV-1-positive serum and, second, with FITC-conjugated rabbit anti-human IgG (Nakashima et al., 1987; Pauwels et al., 1987). The numbers of HIV-1 antigen-positive cells were measured with a CytoACE-150 (Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a 488-nm Argon laser and 10 mW light output. FITC emission signals were collected by using the standard filter set and amplified logarithmically. Ten thousand events per sample were collected, stored, and analyzed.

Assay for HIV binding

The effects of the test compounds on the binding of HIV-1 particles to MT-4 cells were determined by indirect immunofluorescence and laser flow cytometric analysis (Schols et al., 1989). Briefly, MT-4 cells were exposed to HIV-1 stock (100 times the concentration of the culture supernatant of MOLT-4/HTLV-IIIB) in the presence of various concentrations of the test compounds. After incubation at 37°C for 1 h, the cells were washed twice to remove unbound virus. The cells were then processed for indirect immunofluorescence using a human polyclonal anti-HIV-1-positive serum as the first antibody and an FITC-conjugated rabbit anti-human IgG as the second antibody. After immunofluorescence staining, the cells were washed twice with PBS, resuspended in 0.37% paraformaldehyde in PBS, and analyzed by flow cytometry as described above. The binding inhibitory activity ratio (*BI*) was calculated as follows:

$$BI = 1 - \frac{\%\ MF(\mathrm{VC}) - \%\ MF(\mathrm{CC})}{\%\ MF\,(\mathrm{V}) - \%\ MF(\mathrm{C})} \times 100$$

where MF = mean fluorescence; VC = HIV-infected cells treated with compound; C = control cells (not exposed to HIV-1) treated with compound; V = HIV-infected cells without compound; C = control cells (not exposed to HIV-1 and not treated with compound).

Results

Anti-HIV activity of monomeric hydrolyzable tannins

Table 1 summarizes the anti-HIV activity of 21 monomeric hydrolyzable tannins (MW 484–1255). Gemin D (MW 634) (Fig. 1) effectively protected the MT-4 cells from HIV-induced cytopathic effect (CPE). From the titration curve (Fig. 2), the 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of gemin D were calculated to be 2.0 and 26.7 μg/ml, respectively, yielding a selectivity index (SI) of 13 (Table 1). Camelliatannin A (MW 1057) had slightly lower activity (EC_{50} = 7.9 μg/ml, CC_{50} = 46.0 μg/ml, SI = 6). In contrast, no significant anti-HIV activity was found with 19 other compounds including (di-, tri-, tetra-, penta-)galloylglucoses, corilagin, tellimagrandins (I, II), 1,3-digalloyl-4,6-HHDP-glucose, casuarictin, isoterchebin, geraniin, carpinusin, dehydrogeraniin, chebulinic acid, chebulagic acid, coriariin B, remurins (A, B) and isoschimawalin A (Table 1).

Anti-HIV activity of dimeric hydrolyzable tannins

Table 2 summarizes the anti-HIV activity of 39 dimeric hydrolyzable tannins (MW 1571–2282). Nobotanin B (MW 1873) (EC_{50} = 2.4 μg/ml, CC_{50} = 33.7 μg/

TABLE 1

Effects of monomeric hydrolyzable tannins on cytopathic effects induced by HIV infection

Compound	Molecular weight	No. of functional groups			CC_{50} μg(/ml)	EC_{50} (μg/ml)	SI
		Gall	HHDP	Others			
2,3-Digalloylglucose	484	2	0	0	33.8	>50.0	<1
1,2,6-Trigalloylglucose	636	3	0	0	29.7	>50.0	<1
1,2,3,6-Tetragalloylglucose	789	4	0	0	33.9	>50.0	<1
Pentagalloylglucose	941	5	0	0	24.0	>25.0	<1
Gemin D	634	1	1	0	26.7	2.0	13
Corilagin	634	1	1	0	13.1	>25.0	<1
Tellimagrandin I	787	2	1	0	22.9	>25.0	<1
1,3-Digalloyl-4,6-HHDP-glucose	787	2	1	0	23.2	>25.0	<1
Tellimagrandin II	939	3	1	0	29.0	>50.0	<1
Casuarictin	937	1	2	0	20.9	>25.0	<1
Isoterchebin	955	3	0	DH(1)	33.0	>50.0	<1
Geraniin	953	1	1	DH(1)	23.6	>25.0	<1
Carpinusin	953	1	1	DH(1)	18.3	>25.0	<1
Dehydrogeraniin	969	1	0	DH(2)	63.1	>100.0	<1
Chebulinic acid	957	3	0	C(1)	19.5	>25.0	<1
Chebulagic acid	955	1	1	C(1)	19.2	>25.0	<1
Coriariin B	1107	2	1	DG(1)	32.6	>50.0	<1
Remurin A	1107	2	1	DG(1)	28.2	>50.0	<1
Remurin B	955	1	1	DG(1)	29.1	>50.0	<1
Isoschimawalin A	1255	1	0	V(1)L(1)	31.9	>50.0	<1
Camelliatannin A	1057	0	2	0	46.0	7.9	6

Gall, galloyl; HHDP, hexahydroxydiphenoyl; DH, dehydrohexahydroxydiphenoyl; C, chebuloyl; DG, dehydrodigalloyl; V, valoneoyl; L, lactonized valoneoyl.

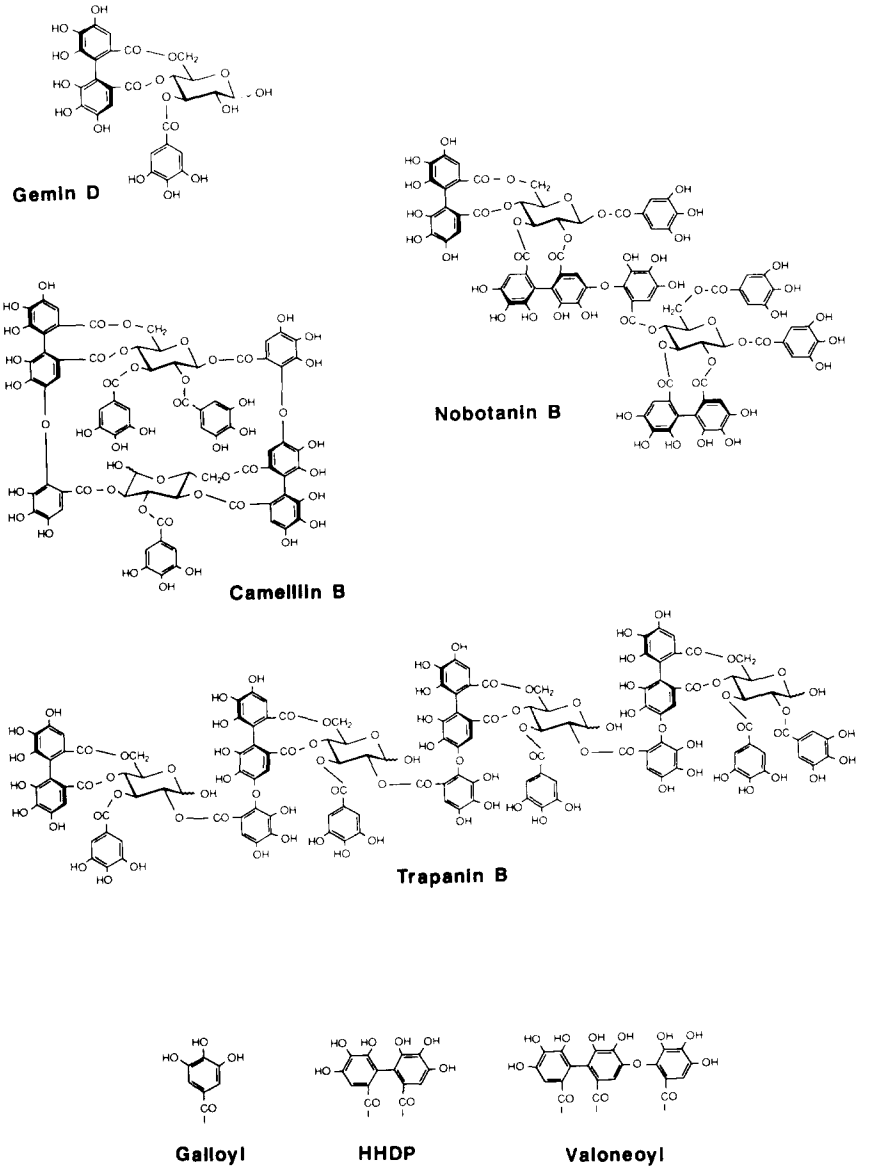

Fig. 1. Structures of gemin D, nobotanin B, camelliin B and trapanin B and their functional groups.

ml, SI = 14) and camelliin B (MW 1721) (EC_{50} = 4.8 μg/ml, CC_{50} = 74.3 μg/ml, SI = 15) (Fig. 1) showed the most potent anti-HIV activity (Fig. 2). It should be noted that all three nobotanins (A, B, F) were effective (SI = 5–14). Slightly weaker activity was found with gemin A, isohirtellin C, tamarixinin A, rugosin D, schimawalin B and euphorbin E (SI = 2–4). However, no significant anti-HIV activity was found with 29 other compounds including agrimoniin,

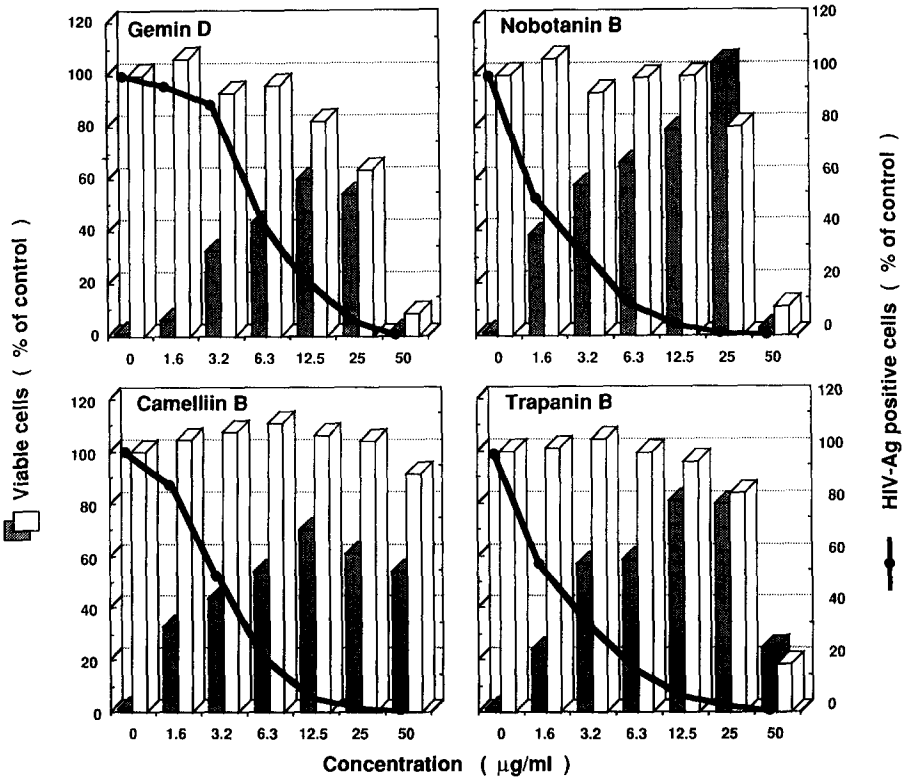

Fig. 2. Inhibition by four hydrolyzable tannins of HIV-induced cytopathic effects and HIV-specific antigen expression. The viable HIV-infected compound-treated MT-4 cells (black bars) and mock-infected compound-treated (open bars) MT-4 cells were expressed as percent of mock-infected and compound-free control cells. The number of HIV-1 antigen-positive cells (solid circles), determined by indirect immunofluorescence and laser flow cytometry, was expressed as percent of virus-infected and compound-free positive control cells.

hirtellins (A–E), coriariin A, rugosin E, coriariins (D, E), desgalloylrugosin F, isorugosins (D, E), cornusiin A, camelliin A, woodfordins (A–C, H, I), euphorbins (A–D, F–H), antidesmin A, and nonacosa-*O*- methylcoriariin A.

Anti-HIV activity of oligomeric hydrolyzable tannins

Table 3 summarizes the anti-HIV activity of 8 oligomeric hydrolyzable tannins (MW 2354–3745). Among 5 trimeric hydrolyzable tannins, nobotanin C (MW 2658) was the most potent inhibitor ($EC_{50} = 3.1$ μg/ml, $CC_{50} = 32.7$ μg/ml, SI = 10). Cornusiin C, trapanin A, oenothein A were slightly effective (SI = 2), and woodfordin D was totally inactive (SI < 1).

Among 3 tetrameric hydrolyzable tannins, trapanin B (MW 3140) (Hatano et al., 1990) (Fig. 1) was the most active ($EC_{50} = 2.7$ μg/ml, $CC_{50} = 39.1$ μg/ml, SI = 14) (Fig. 2). Nobotanin K had slightly lower activity (SI = 7), and woodfordin F was totally inactive (SI < 1).

TABLE 2

Effects of dimeric hydrolyzable tannins on cytopathic effects induced by HIV infection

Compound	Molecular weight	No. of functional groups			CC_{50} (μg/ml)	EC_{50} (μg/ml)	SI
		Gall	HHDP	Others			
Agrimoniin	1871	0	4	DG(1)	41.3	>50.0	<1
Gemin A	1873	2	3	DG(1)	19.8	7.7	3
Hirtellin A	1875	4	2	DG(1)	56.8	>100.0	<1
Hirtellin B	1873	3	2	He(1)	54.3	>100.0	<1
Hirtellin C	1873	2	2	DG(1)I(1)	39.9	>50.0	<1
Hirtellin D	1571	3	1	He(1)	58.6	>100.0	<1
Hirtellin E	1571	2	1	DG(1)I(1)	59.0	>100.0	<1
Isohirtellin C	1873	2	2	DG(2)	75.5	25.0	3
Tamarixinin A	1721	2	2	He(1)	64.3	21.7	3
Coriariin A	1875	4	2	DG(1)	37.7	>50.0	<1
Rugosin D	1875	5	1	V(1)	24.7	11.8	2
Rugosin E	1723	4	1	V(1)	30.6	>50.0	<1
Coriariin D	1891	4	0	V(2)	50.5	>100.0	<1
Coriariin E	1571	3	1	V(1)	49.8	>50.0	<1
Desgalloylrugosin F	1721	2	2	V(1)	26.0	>50.0	<1
Isorugosin D	1875	5	1	V(1)	70.3	>100.0	<1
Isorugosin E	1723	4	1	V(1)	30.2	>50.0	<1
Cornusiin A	1571	3	1	V(1)	34.6	>50.0	<1
Camelliin A	1569	1	2	V(1)	44.4	>50.0	<1
Woodfordin A	1725	6	0	V(1)	58.6	>100.0	<1
Woodfordin B	1723	4	1	V(1)	42.0	>50.0	<1
Woodfordin H	1871	2	1	V(1)L(1)	46.5	>50.0	<1
Schimawalin B	1721	3	0	V(1)L(1)	52.8	11.8	4
Nobotanin A	1721	2	2	V(1)	33.6	7.0	5
Nobotanin B	1873	3	2	V(1)	33.7	2.4	14
Nobotanin F	1873	3	2	V(1)	34.8	4.5	8
Euphorbin A	1891	5	0	V(1)DH(1)	29.3	>50.0	<1
Euphorbin B	1891	5	0	V(1)DH(1)	28.6	>50.0	<1
Euphorbin C	1887	2	1	E(1)DH(1)	33.4	>50.0	<1
Euphorbin D	1889	4	0	E(1)DH(1)	34.6	24.4	1
Euphorbin E	1885	2	0	DE(1)DH(2)	34.8	13.5	3
Euphorbin F	1889	3	1	V(1)DH(1)	33.2	>50.0	<1
Euphorbin G	1889	3	1	V(1)DH(1)	37.4	>50.0	<1
Euphorbin H	1571	3	1	V(1)	33.5	>50.0	<1
Antidesmin A	1889	3	1	V(1)DH(1)	32.3	>50.0	<1
Woodfordin C	1721	3	0	V(2)	50.8	>100.0	<1
Woodfordin I	1737	2	0	V(1)W(1)	96.7	>100.0	<1
Camelliin B	1721	3	0	V(2)	74.3	4.8	15
Nonacosa-*O*-methylcoriariin A	2282	[4][a]	[2][a]	DG[1][a]	>100.0	>100.0	<1

[a] Methylated acyl groups. He, hellinoyl; I, isodehydrodigalloyl; E, euphorbinoyl; DE, dehydroeuphorbinoyl; W, woodfordinoyl.

Anti-HIV activity of condensed tannins and other polyphenols

Table 4 summarizes the anti-HIV activity of condensed tannins (MW 883–1764) and other polyphenols (MW 170–719). Condensed tannins such as (−)-epicatechin 3-*O*-gallate (ECG)-dimer, ECG-trimer and ECG-tetramer, and related polyphenols had very low anti-HIV activity (SI = 1–2).

TABLE 3

Effects of oligomeric hydrolyzable tannins on cytopathic effects induced by HIV infection

Compound	Molecular weight	No. of functional groups			CC_{50} (μg/ml)	EC_{50} (μg/ml)	SI
		Gall	HHDP	Others			
Trimeric hydrolyzable tannins							
Nobotanin C	2658	4	2	V(2)	32.7	3.1	10
Cornusiin C	2356	4	1	V(2)	40.5	24.5	2
Trapanin A	2508	5	1	V(2)	36.6	14.8	2
Oenothein A	2354	3	1	V(1)W(1)	48.2	20.2	2
Woodfordin D	2506	4	1	V(1)W(1)	57.3	>100.0	<1
Tetrameric hydrolyzable tannins							
Nobotanin K	3745	5	3	V(3)	38.4	5.8	7
Trapanin B	3140	5	1	V(3)	39.1	2.7	14
Woodfordin F	3138	4	1	V(2)W(1)	60.6	>100.0	<1

TABLE 4

Effects of condensed tannins and other polyphenols on cytopathic effects induced by HIV infection

Compound	Molecular weight	No. of functional groups			CC_{50} (μg/ml)	EC_{50} (μg/ml)	SI
		Gall	HHDP	Others			
Condensed tannins and related polyphenols							
(+)-Catechin	290	0	0	0	>100.0	>100.0	<1
(−)-Epicatechin	290	0	0	0	72.5	>100.0	<1
EGC	306	0	0	0	14.6	>25.0	<1
ECG	442	1	0	0	36.2	>50.0	<1
EGCG	458	1	0	0	16.7	>25.0	<1
ECG-dimer	883	2	0	0	52.5	>50.0	<1
ECG-trimer	1323	3	0	0	60.6	>100.0	<1
ECG-tetramer	1764	4	0	0	55.5	22.5	2
Other polyphenols							
Gallic acid	170	1	0	0	4.3	>6.3	<1
Methyl gallate	184	1	0	0	9.5	>12.5	<1
Ethyl gallate	198	1	0	0	8.6	>12.5	<1
n-Propyl gallate	212	1	0	0	11.4	>12.5	<1
n-Butyl gallate	226	1	0	0	14.4	>25.0	<1
Lauryl gallate	338	1	0	0	1.3	>3.1	<1
Stearyl gallate	423	1	0	0	1.4	>3.1	<1
Ellagic acid	302	0	1	0	80.0	>100.0	<1
Rosmarinic acid	360	0	0	0	28.3	>50.0	<1
Rabdosiin	719	0	0	0	29.2	>50.0	<1
Taxifolin apioside	434	0	0	0	54.3	>100.0	<1

EGC, (−)-epigallocatechin; ECG, (−)-epicatechin 3-*O*-gallate [=3-galloyl-(−)-epicatechin]; EGCG, (−)-epigallocatechin 3-*O*-gallate; ECG-dimer, 3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin; ECG-trimer, 3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin; ECG-tetramer, 3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin-(4β-8)-3-*O*-galloylepicatechin.

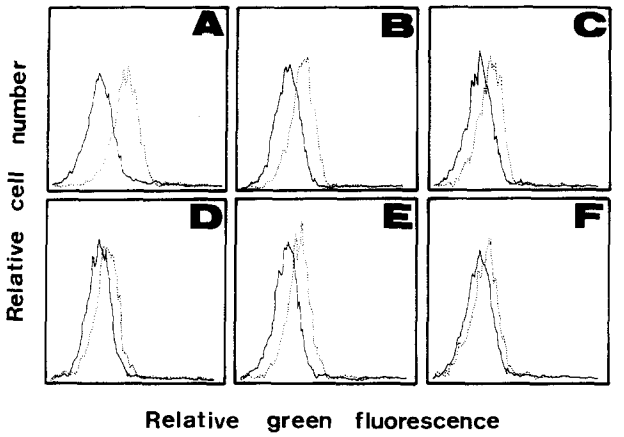

Fig. 3. Inhibition by four hydrolyzable tannins of HIV-1 binding to MT-4 cells. The solid line represents nonspecific fluorescence of uninfected MT-4 cells. The dotted line represents fluorescence of MT-4 cells which were exposed to HIV-1 virions. MT-4 cells were incubated in the absence of test compound (A) or in the presence of gemin D (3.13 μg/ml) (B), nobotanin B (3.13 μg/ml) (C), camelliin B (3.13 μg/ml) (D), trapanin B (3.13 μg/ml (E), 12.5 μg/ml (F)).

Other polyphenols, such as gallic acid, its 6 derivatives (methyl, ethyl, n-propyl, n-butyl, lauryl, stearyl gallate), ellagic acid, rosmarinic acid, rabdosiin and taxifolin apioside were also inactive.

Effect on HIV antigen expression

The potent anti-HIV compounds (gemin D, nobotanin B, camelliin B and trapanin B) reduced the expression of HIV antigen in a dose-dependent fashion (Fig. 2). Their 50% inhibitory concentrations were calculated to be 6.0, 1.8, 3.1 and 2.1 μg/ml, respectively.

Inhibition of HIV adsorption

Fig. 3 shows that gemin D, nobotanin B, camelliin B and trapanin B inhibited the HIV-1 binding to MT-4 cells. From the dose response curve

TABLE 5

Inhibitory effects of gemin D, nobotanin B, camelliin B and trapanin B on HIV-1 binding to MT-4 cells

Concentration (μg/ml)	Binding-inhibitory activity (%)			
	Gemin D	Nobotanin B	Camelliin B	Trapanin B
0.78	1.9	42.6	43.0	44.7
1.56	0.7	73.6	62.0	52.1
3.13	39.1	84.0	66.3	61.9
6.25	70.5	88.5	64.7	80.6
12.5	67.4	81.0	60.9	90.5

These data were obtained from experiments similar to those presented in Fig. 3.

(Table 5), their 50% inhibitory concentrations were calculated to be 4.0, 0.9, 1.0 and 1.3 μg/ml, respectively.

Discussion

The present report revealed that several hydrolyzable tannins (gemin D, nobotanin B, camelliin B, trapanin B), but not condensed tannins nor related polyphenols of lower molecular weight, have potent anti-HIV activity. These potent compounds generally contain HHDP and/or valoneoyl group(s), but none has dehydrohexahydroxydiphenoyl, chebuloyl, dehydrodigalloyl, isodehydrodigalloyl, lactonized valoneoyl, hellinoyl, euphorbinoyl, dehydroeuphorbinoyl, or woodfordinoyl groups (Tables 1–3). However, there are several compounds that exhibit only low anti-HIV activity, despite the presence of HHDP and/or valoneoyl group(s) in each molecule. Anti-HIV activity then seems to be correlated with the whole structure of each polyphenolic compound, most probably with the specific stereostructure induced by the presence of each polyphenolic functional group, such as HHDP and the valoneoyl group.

The anti-HIV activity of the hydrolyzable tannins, as reflected by SI values, seems to increase with the number of repeating units: monomeric < dimeric < trimeric < tetrameric (Tables 1–3). This suggests dependence of anti-HIV activity on the polyanionic character of the compounds. Dependence of anti-HIV activity on molecular weight has also been demonstrated with a series of heterogeneous polymers derived from aurintricarboxylic acid (Cushman et al., 1991) and polyhydroxycarboxylates derived from phenol-like compounds (Schols et al., 1991). Furthermore, several high molecular weight lignified materials, which have heterogeneous structures composed of polymerized phenylpropanoid and polysaccharides (Kirk and Obst, 1988), have shown potent anti-HIV activity (Lai et al., 1990; Suzuki et al., 1989).

It has recently been reported that the anti-HIV activity of some gallo- and ellagitannins is not necessarily associated with their inhibition of reverse transcriptase activity (Nonaka et al., 1990). Also, inhibition of herpes simplex virus infection by tannins has been reported (Fukuchi et al., 1989). The present report suggests that the anti-HIV activity of several hydrolyzable tannins might be mediated, at least, in part by inhibition of HIV adsorption to the cells (Fig. 3). However, it should be noted that tannins, at a concentration as high as 12.5 μg/ml, did not completely inhibit HIV binding (Table 5).

We recently found that dimeric hydrolyzable tannins are potent inhibitors of poly(ADP-ribose) glycohydrolase (Tsai et al., 1991), an enzyme that plays a regulatory role in gene transcription (Tanuma et al., 1983). These tannins also inhibit the expression of mRNA of mouse mammary tumor virus (Tsai et al., submitted). Thus, the broad spectrum of biological effects of tannins remains to be further delineated.

Acknowledgements

We thank Dr. A. Simpson for help with the manuscript. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

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