Manstein, D. J., Pai, E. F., Schopfer, L. M., & Massey, V. (1986) *Biochemistry 25*, 6807–6816.
Massey, V., & Ghisla, S. (1974) *Ann. N.Y. Acad. Sci. 227*, 46–465.
Massey, V., Gibson, Q. H., & Veeger, C. (1960) *Biochem. J. 77*, 341–351.
Matthews, R. G., & Williams, C. H., Jr. (1976) *J. Biol. Chem. 251*, 3956–3964.
Matthews, R. G., Ballou, D. P., & Williams, C. H., Jr. (1979) *J. Biol. Chem. 254*, 4974–4981.
Miller, S. M., Ballou, D. P., Massey, V., Williams, C. H., Jr., & Walsh, C. T. (1986) *J. Biol. Chem. 261*, 2081–2084.
Miller, S. M., Moore, M. J., Massey, V., Williams, C. H., Jr., Distefano, M. D., Ballou, D. P., & Walsh, C. T. (1989) *Biochemistry 28*, 1194–1205.
Moore, M. J. (1989) Ph.D. Thesis, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA.
Moore, M. J., & Walsh, C. T. (1989) *Biochemistry 28*, 1183–1194.
O'Donnell, M. E., & Williams, C. H., Jr. (1984) *J. Biol. Chem. 259*, 2243–2251.
Pai, E. F., & Schulz, G. E. (1983) *J. Biol. Chem. 258*, 1752–1757.
Sahlman, L., Lambeir, A.-M., Lindskog, S., & Dunford, H. B. (1984) *J. Biol. Chem. 259*, 12403–12408.
Sahlman, L., Lambeir, A., & Lindskog, S. (1986) *Eur. J. Biochem. 156*, 479–488.
Sandstrom, A., & Lindskog, S. (1988) *Eur. J. Biochem. 173*, 411–415.
Schultz, P. G., Au, K. G., & Walsh, C. T. (1985) *Biochemistry 24*, 6840–6848.
Searls, R. L., Peters, J. M., & Sanadi, D. R. (1961) *J. Biol. Chem. 236*, 2317–2322.
Seidman, M. (1985) *BMBiochemica 2* (5), 10.
Strickland, S., Palmer, G., & Massey, V. (1975) *J. Biol. Chem. 250*, 4048–4052.
Thorpe, C., & Williams, C. H., Jr. (1976) *J. Biol. Chem. 251*, 7726–7728.
Thorpe, C., & Williams, C. H., Jr. (1981) *Biochemistry 20*, 1507–1513.
Venkataram, U. V., & Bruice, T. C. (1984) *J. Am. Chem. Soc. 106*, 5703–5709.
Vervoort, J., Müller, F., Lee, J., van den Berg, W. A. M., & Moonen, C. T. W. (1986) *Biochemistry 25*, 8062–8067.
Walker, W. H., Hemmerich, P., & Massey, V. (1970) *Eur. J. Biochem. 13*, 258–266.
Williams, C. H., Jr. (1976) *Enzymes (3rd Ed.) 13*, 89–173.
Yokoe, I., & Bruice, T. C. (1975) *J. Am. Chem. Soc. 97*, 450–451.

Differential Inhibitory Effects of Some Catechin Derivatives on the Activities of Human Immunodeficiency Virus Reverse Transcriptase and Cellular Deoxyribonucleic and Ribonucleic Acid Polymerases

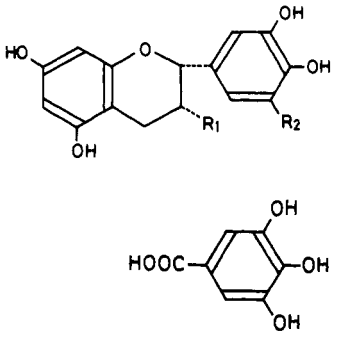
Hideo Nakane and Katsuhiko Ono*
Laboratory of Viral Oncology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan
Received July 26, 1989; Revised Manuscript Received November 2, 1989

ABSTRACT: The two components of *Camellia sinensis* (tea plant) [i.e., (−)-epicatechin gallate and (−)-epigallocatechin gallate] were found to differentially inhibit the activities of reverse transcriptase and cellular DNA and RNA polymerases. Under the assay conditions optimized for each enzyme species, the strongest inhibition by these compounds was observed with reverse transcriptase. The concentrations of (−)-epicatechin gallate and (−)-epigallocatechin gallate required for 50% inhibition of the activity of human immunodeficiency virus (HIV) reverse transcriptase were in the range of 0.01–0.02 μg/mL. On the other hand, neither (−)-epicatechin, (−)-epigallocatechin, nor gallic acid, the constituents of (−)-epicatechin gallate and (−)-epigallocatechin gallate, was inhibitory to the activity of HIV reverse transcriptase at concentrations up to 1 μg/mL. The mode of inhibition of reverse transcriptase and other DNA polymerases by these compounds was competitive with respect to the template-primer, whereas the mode of inhibition of RNA polymerase was competitive with respect to the nucleotide substrate. The K_i values of HIV reverse transcriptase for (−)-epicatechin gallate and (−)-epigallocatechin gallate were determined to be 7.2 and 2.8 nM, respectively, which are smaller by 1–2 orders of magnitude than the K_i's of other DNA and RNA polymerases for these compounds.

Since AIDS[1] was found to be caused by a retrovirus, designated as HIV, HIV-associated reverse transcriptase has been considered to be one of the appropriate targets for chemotherapeutic approaches toward AIDS. Various reverse transcriptase inhibitors have already been reported to exert anti-HIV effects, e.g., suramin (Mitsuya et al., 1984; Broder et al., 1985), HPA23 (Rozenbaum et al., 1985), AZT (Mitsuya et al., 1985; Yarchoan et al., 1986), and DDC (Mitsuya & Broder, 1986). However, most of the antiretroviral agents that inhibit reverse transcriptase also inhibit cellular DNA polymerases. Typical examples are suramin and HPA23, which inhibit both reverse transcriptase and cellular DNA polymerases (Ono et al., 1988a,b). This explains at least part of the side effects observed upon administration of these

[1] Abbreviations: AIDS, acquired immune deficiency syndrome; ARC, AIDS-related complex; HIV, human immunodeficiency virus; RLV, Rauscher murine leukemia virus; suramin, hexasodium *N,N'*-bis[3-[[[4-methyl-3-[[(4,6,8-trisulfo-1-naphthyl)amino]carbonyl]phenyl]amino]-carbonyl]phenyl]carbamide; HPA23, hexaoctacontaoxononaantimonateheneicosatungstate(19−) heptadecaammonium salt; AZT and AZTTP, 3'-azido-3'-deoxythymidine and its 5'-triphosphate; DDC, 2',3'-dideoxycytidine; dNTP, 2'-deoxynucleoside 5'-triphosphate.

0006-2960/90/0429-2841$02.50/0



gallic acid

	R_1	R_2
(−)-Epicatechin	OH	H
(−)-Epigallocatechin	OH	OH
(−)-Epicatechin gallate	gallic acid ester	H
(−)-Epigallocatechin gallate	gallic acid ester	OH

FIGURE 1: Structural formulas of various catechins.

anti-HIV agents to AIDS or ARC patients.

To find more selective but less toxic compounds, we have been looking for natural products inhibitory to reverse transcriptase. It was reported that some plant extracts (Ono et al., 1989b,c) as well as a flavonoid (Ono et al., 1989a) had inhibitory effects on the activities of reverse transcriptases from RLV and HIV. We have successively tested various flavonoids and their related compounds for inhibition of HIV reverse transcriptase. Here we report that (−)-epicatechin gallate and (−)-epigallocatechin gallate, the components of *Camellia sinensis* (tea plant), were strong inhibitors of HIV reverse transcriptase. In addition, this paper describes in detail the inhibitory effects and the inhibition mode of these compounds on the activities of various nucleotide-polymerizing enzymes.

Materials and Methods

Chemicals. The sources of materials used in this work were as follows: (−)-epicatechin gallate, (−)-epigallocatechin gallate, (−)-epicatechin, and (−)-epigallocatechin (Figure 1) purified from *C. sinensis* with HPLC from Mitsui Norin Co. Ltd. Food Research Laboratories (Fujieda, Japan); gallic acid from Katayama Chemical Industries Co. Ltd. (Osaka, Japan); [^{3}H]dNTPs and [^{3}H]GTP from Amersham International (Amersham, England); unlabeled nucleotides, $(rA)_n$, $(dC)_n$, and $(dA)_{12-18}$ from P-L Biochemicals, Inc. (Milwaukee, WI); activated calf thymus DNA from Worthington Biochemical Corp. (Freehold, NJ); DEAE-cellulose paper discs (DE81, diameter 23 mm) from Whatman Ltd. (Springfield Mill, Maidstone, Kent, England).

Reverse Transcriptases. HIV reverse transcriptase was purified from *Escherichia coli* harboring an expression plasmid for the precise coding sequence of the enzyme. The purified enzyme was a generous gift from Dr. S. H. Wilson, NIH. RLV was obtained from the culture medium of an established virus-producing cell line, R-17, and reverse transcriptase was purified on a DEAE-Sephadex A-50 column as previously described (Nakajima et al., 1974).

DNA and RNA Polymerases. DNA polymerases α, β, and γ were purified from KB III cells, as previously described for DNA polymerases α (Matsukage et al., 1976), β (Ono et al., 1979), and γ (Yamaguchi et al., 1980) with some modifications. Terminal deoxynucleotidyltransferase was purified from calf thymus, as previously described (Okamura et al., 1978) with some modifications. Highly purified preparations of *E. coli* DNA and RNA polymerases were purchased from P-L Biochemicals, Inc. (Milwaukee, WI).

Assays for Reverse Transcriptase and DNA and RNA Polymerase Activities. Reverse transcriptase activity was measured with $(rA)_n$·$(dT)_{12-18}$ as the template-primer under the optimized reaction conditions specified for each of the HIV and RLV reverse transcriptases. The reaction mixture contained the following components: 50 mM Tris-HCl, pH 8.0; 2 μg/mL $(rA)_n$·$(dT)_{12-18}$ (1:1); 10 μM [^{3}H]dTTP (400 cpm/pmol); 5 mM dithiothreitol; 50 mM KCl; 15% (v/v) glycerol; and 0.2 mM $MnCl_2$ for RLV reverse transcriptase and 5 mM $MgCl_2$ for HIV reverse transcriptase.

Other DNA and RNA polymerase activities were measured under the reaction conditions described previously (Ono et al., 1989c).

The reaction (50-μL total volume) was started by adding 5 μL of enzyme, the reaction mixture was incubated at 37 °C for 30 min, and the reaction was stopped by adding 20 μL of 0.2 M EDTA and immersing the mixture in ice. Then a 50-μL aliquot of the mixture was transferred to a DE81 filter paper disc and processed for radioactivity counting as previously described (Lindell et al., 1967).

Results

Effects of Various Catechins on the Activities of Reverse Transcriptase and DNA and RNA Polymerases. The effects of (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate on the activities of various nucleotide-polymerizing enzymes were examined under the conditions described under Materials and Methods. Both (−)-epicatechin gallate and (−)-epigallocatechin gallate were found to strongly inhibit the activities of RLV reverse transcriptase and DNA polymerases α and β (Figure 2, parts A and B). *E. coli* DNA polymerase I and RNA polymerase were also inhibited strongly by (−)-epigallocatechin gallate and moderately by (−)-epicatechin gallate. DNA polymerase γ was moderately sensitive to inhibition by both compounds. However, terminal deoxynucleotidyltransferase was insensitive to inhibition by these compounds at concentrations up to 1 μg/mL (Figure 2A,B). On the other hand, (−)-epicatechin and (−)-epigallocatechin, the constituents of these inhibitors, did not show any remarkable inhibitory effects on the activities of these DNA and RNA polymerases (data not shown). The strongest inhibition by these two compounds was observed with HIV reverse transcriptase, because 50% inhibition was obtained at inhibitor concentrations of 0.01–0.02 μg/mL (Figure 2C). Neither (−)-epicatechin, (−)-epigallocatechin, nor gallic acid was inhibitory to HIV reverse transcriptase at concentrations up to 0.1 μg/mL (Figure 2C).

Analysis of the Mode of Inhibition of Various DNA and RNA Polymerases by (−)-Epicatechin Gallate and (−)-Epigallocatechin Gallate. The inhibition mode of (−)-epicatechin gallate and (−)-epigallocatechin gallate was analyzed kinetically by changing the concentration of either the template-primer or the triphosphate substrate in the presence of various concentrations of each of these two inhibitors. The data obtained were treated by double-reciprocal plotting, and the results are summarized in Table I; some typical examples are explained as follows. The mode of inhibition of DNA polymerases including reverse transcriptase by either of these two compounds was competitive with respect to the template-primer except for HIV reverse transcriptase (mixed type) and noncompetitive with respect to the dTTP substrate (Table I). For example, both of the compounds inhibited RLV reverse transcriptase activity by competing with $(rA)_n$·$(dT)_{12-18}$ but not with the dTTP substrate. In the case of HIV reverse

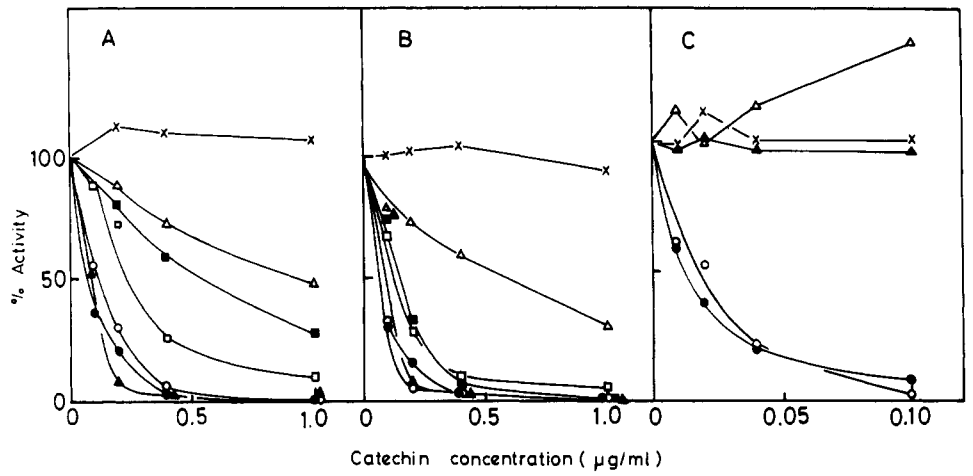


FIGURE 2: Effects of various catechins on the activities of RLV and HIV reverse transcriptases and various DNA and RNA polymerases. The activities were measured under the conditions described under Materials and Methods, in the presence of various concentrations of catechins or gallic acid as indicated in the figure, by determining the incorporation of the radiolabeled triphosphate substrate. (A) (−)-Epicatechin gallate. (B) (−)-Epigallocatechin gallate. The polymerases tested and the symbols used in (A) and (B) are as follows: RLV reverse transcriptase (●), DNA polymerase α (○), DNA polymerase β (▲), DNA polymerase γ (△), terminal deoxynucleotidyltransferase (×), *E. coli* DNA polymerase I (■), and RNA polymerase (□). The specific radioactivities of [^{3}H]dNTP and [^{3}H]GTP were 6000 (△), 1000 (○), 400 (●, ▲, ×, ■), and 16 (□) cpm/pmol. The 100% values (pmol) were 59.6 (●), 22.7 (○), 8.9 (▲), 1.45 (△), 231.3 (×), 65.0 (■), and 20.8 (□). (C) Effects on the activity of HIV reverse transcriptase. The activity was measured by determining the incorporation of [^{3}H]dTMP (400 cpm/pmol) with $(rA)_n$·$(dT)_{12-18}$ as the template-primer. The symbols used in (C) were as follows: (−)-epicatechin gallate (●), (−)-epigallocatechin gallate (○), (−)-epicatechin (▲), (−)-epigallocatechin (△), and gallic acid (×). The 100% value (pmol) was 16.2.

Table I: Characterization of Inhibition of Reverse Transcriptase and Various DNA and RNA Polymerases by (−)-Epicatechin Gallate and (−)-Epigallocatechin Gallate

		(−)-epicatechin gallate		(−)-epigallocatechin gallate		
DNA and RNA polymerase	variable substrate	mode	K_i (nM)	mode	K_i (nM)	K_m[a]
reverse transcriptase						
HIV	$(rA)_n$·$(dT)_{12-18}$ (1:1)	M[b]	7.2	M	2.8	0.8
	dTTP	NC[c]		NC		3.0
RLV	$(rA)_n$·$(dT)_{12-18}$ (1:1)	C[d]	47.5	C	34.9	0.4
	dTTP	NC		NC		15.5
eucaryotic						
α	activated DNA	C	181	C	116	162
	dTTP	NC		NC		0.9
β	$(rA)_n$·$(dT)_{12-18}$ (1:2)	C	23.7	C	71.1	23.2
	dTTP	NC		NC		61.3
γ	$(rA)_n$·$(dT)_{12-18}$ (10:1)	C	298	C	286	69.6
	dTTP	NC		NC		1.7
E. coli RNA polymerase	$(dC)_n$	NC → C[e]		NC → C		2.6
	GTP	C	323	C	176	450

[a] The K_m values are expressed in μg/mL for the template (when synthetic template-primers were used) and the template-primer (activated DNA) and μM for the nucleotide substrates. [b] M: mixed type. [c] NC: noncompetitive type. [d] C: competitive type. [e] NC → C: the mode of inhibition changed from noncompetitive to competitive type by increasing the concentration of $(dC)_n$.

transcriptase, however, the inhibition mode of (−)-epicatechin gallate was not purely competitive with respect to $(rA)_n$·$(dT)_{12-18}$ and noncompetitive with respect to dTTP. (−)-Epigallocatechin gallate inhibited the activity of DNA polymerase α competitively with respect to the template-primer (activated DNA) and noncompetitively with respect to the dTTP substrate. A similar inhibition mode was also obtained with DNA polymerase β (Table I).

On the other hand, the mode of inhibition of RNA polymerase showed complexity when the inhibition was analyzed with varying concentrations of $(dC)_n$ template. When the concentrations of $(dC)_n$ were relatively low (i.e., 1–10 μg/mL), the mode of inhibition by (−)-epicatechin gallate was noncompetitive with respect to the template. When analyzed at higher $(dC)_n$ concentrations between 10 and 100 μg/mL, however, the inhibition mode changed to purely competitive with respect to $(dC)_n$ template. On the other hand, the inhibition mode of (−)-epicatechin gallate was always competitive with respect to GTP substrate over the entire concentration range examined. Similar results were also obtained with (−)-epigallocatechin gallate (Table I).

Determination of the Kinetic Constants of (−)-Epicatechin Gallate and (−)-Epigallocatechin Gallate. The inhibition potentials of (−)-epicatechin gallate and (−)-epigallocatechin gallate on the activities of reverse transcriptase and various DNA and RNA polymerases were evaluated by determining the inhibition constants (K_i's) by replotting (Dixon plot) the data in the previous section; the K_i values are summarized in Table I. As seen in this table, the smallest K_i value was obtained with HIV reverse transcriptase, supporting the fact that the strongest inhibition by these compounds was observed with this enzyme (Figure 2C).

Discussion

As seen in Figure 2, (−)-epicatechin gallate and (−)-epigallocatechin gallate strongly inhibit the activities of various DNA and RNA polymerases except terminal deoxynucleotidyltransferase (Figure 2, parts A and B). However, the strongest inhibition by these compounds was observed with HIV reverse transcriptase, since the concentration of these

compounds required for 50% inhibition (IC_{50}) of the enzyme activity was about 0.02 μg/mL (0.045 μM) (Figure 2C). This IC_{50} value was less than one-fifth of those for other DNA polymerases. The smallest K_i values of HIV reverse transcriptase for these compounds (Table I) also support the strongest inhibitory effects of these compounds on this enzyme. These results indicate a possibility that (−)-epicatechin gallate and (−)-epigallocatechin gallate exert selective inhibitory effects on the reverse transcriptase if appropriate concentrations of the compounds are chosen. The IC_{50} of AZTTP, a well-known inhibitor for HIV reverse transcriptase, has been estimated to be 0.02 μM under the same reaction conditions (Ono et al., unpublished data). (−)-Epicatechin gallate and (−)-epigallocatechin gallate have thus proved to be as strong inhibitors of HIV reverse transcriptase as AZTTP.

In order to clarify the structure–activity relationships of this class of compounds, the constituents of (−)-epicatechin gallate and (−)-epigallocatechin gallate were tested for inhibition of the enzyme activities. Neither (−)-epicatechin nor (−)-epigallocatechin was inhibitory to any of the DNA and RNA polymerases tested (data not shown). Also, gallic acid was virtually ineffective against the activity of HIV reverse transcriptase (Figure 2C). These results indicate that the whole structure of catechin with the 3-galloyl group is indispensable for the expression of inhibitory activity against various DNA and RNA polymerases. As shown in Figure 2, however, the inhibition by (−)-epigallocatechin gallate is slightly stronger than that by (−)-epicatechin gallate except for DNA polymerases β and γ. The smaller K_i values of (−)-epigallocatechin gallate than those of (−)-epicatechin gallate support this slight difference (Table I). This difference between the two compounds seems to be due to the presence [(−)-epigallocatechin gallate] or absence [(−)-epicatechin gallate] of a hydroxyl group on the 5′-position of the B-ring in the catechin moiety. Both compounds have many other hydroxyl groups; therefore, further studies with various analogues in which the number and/or the positions of hydroxyl groups are varied should be helpful in elucidating their structure–activity relationships.

With most of the DNA polymerases, both (−)-epicatechin gallate and (−)-epigallocatechin gallate exert their inhibitory action by competing with the template-primer on the enzyme molecule (Table I). The results are similar to those of 5,6,7-trihydroxyflavone (baicalein), which inhibits reverse transcriptase activity by competing with the template-primer (Ono et al., 1989a). However, the inhibition of HIV reverse transcriptase activity by these catechin derivatives was of a mixed type with respect to the template-primer, suggesting that the structure of the active center of HIV reverse transcriptase is different from that of the RLV enzyme.

It is, however, still unclear whether these compounds bind to the template-primer itself or to the template-primer binding site of the enzyme or to both. We observed, however, that the addition of 400 μg/mL bovine serum albumin into the reaction mixture greatly reduced the inhibitory effects of these compounds on RLV reverse transcriptase (data not shown), suggesting strong affinities of these compounds for protein. Furthermore, only a slight inhibition of DNA polymerase γ (Figure 2) observed under reaction conditions similar to those of reverse transcriptase argues against a possibility that the major mechanism of inhibition is binding of these compounds to the template-primer, followed by inactivation of the priming activity. From these results, it seems most likely that any of these catechin inhibitors bind to the enzyme protein itself and inhibit the activity by interfering with binding of the template-primer to the enzyme.

Inhibition of RNA polymerase by these catechin derivatives showed some complexity, because noncompetitive-type inhibition changed to competitive type by increasing the concentration of $(dC)_n$ template (Table I). These biphasic kinetics could be explained by the presence of a negative cooperativity among multiple template-primer binding sites of the enzyme. A similar phenomenon was also reported for the inhibition of human DNA polymerase α by Evans blue (Nakane et al., 1988) as well as for the inhibition of murine DNA polymerase α by aphidicolin (Ono et al., 1983). A negative cooperativity was observed when the concentration of the template-primer (activated DNA) was increased in the former case and when the species of the dNTP substrate was changed in the latter case. Thus, the activity of RNA polymerase is, like the inhibition of DNA polymerase α by Evans blue or aphidicolin, regulated allosterically in the presence of the catechin inhibitors.

Both (−)-epicatechin gallate and (−)-epigallocatechin gallate are natural products occurring in green tea. These compounds posses a galloyl group and, therefore, belong to a category of tannin. The contribution of hydroxyl groups to the inhibitory activity seems to be clear. It may thus be possible to enhance the potency of this class of compounds further as inhibitors of reverse transcriptase by modifying their chemical structure, i.e., number and position (and/or distribution) of the hydroxyl groups.

Finally, it should be noted that the K_i value of HIV reverse transcriptase for (−)-epigallocatechin gallate is considerably smaller (2.8 nM) than those of other cellular DNA and RNA polymerases (Table I), indicating a high selectivity of this compound for this viral enzyme.

It was unfortunate, however, that a test of (−)-epicatechin gallate and (−)-epigallocatechin gallate in an intact cell culture system with MT-4 cells revealed cytotoxic effects of these compounds at concentrations that did not inhibit HIV-1-induced cytopathogenicity in these cells (Harada et al., 1985). The 50% cytotoxic doses, based on the reduction of viability of mock-infected MT-4 cells, were 31 and 4.6 μg/mL for (−)-epicatechin gallate and (−)-epigallocatechin gallate, respectively. The results may imply that these catechin derivatives are toxic substances to the cultured T-lymphocytes. Alternatively, the catechin derivatives might have difficulties in accessing their target, reverse transcriptase, which resides inside the virus-infected cell. Some structural modifications of these compounds to minimize the toxicity and to enhance the accessibility may resolve the problems.

Acknowledgments

We are grateful to Dr. M. Baba, Fukushima Medical College, for his help in MTT assay to determine the cellular toxicities (CD_{50} values) of the catechin derivatives. We are also grateful to Prof. Y. Ose of Gifu Pharmaceutical University for his useful suggestions. We thank S. Shinmura for preparing the manuscript.

Supplementary Material Available

Figures of the kinetic analyses (double-reciprocal plots) of the inhibition of RLV and HIV reverse transcriptases and *E. coli* RNA polymerase by (−)-epicatechin gallate and of the inhibition of DNA polymerase α by (−)-epigallocatechin gallate (1 page). Ordering information is given on any current masthead page.

References

Broder, S., Yarchoan, R., Collins, J. M., Lane, H. C., Markham, P. D., Klecker, R. W., Redfield, R. R., Mitsuya,

H., Hoth, D. F., Gelman, E., Groopman, J. E., Resnick, L., Gallo, R. C., Myers, C. E., & Fauci, A. S. (1985) *Lancet ii*, 627–630.
Harada, S., Koyanagi, Y., & Yamamoto, N. (1985) *Science (Washington, D.C.) 229*, 563–566.
Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G., & Rutter, M. J. (1967) *Science (Washington, D.C.) 170*, 447–449.
Matsukage, A., Sivarajan, M., & Wilson, S. H. (1976) *Biochemistry 15*, 5305–5314.
Mitsuya, H., & Broder, S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1911–1915.
Mitsuya, H., Popovic, M., Yarchoan, R., Matsushita, S., Gallo, R. C., & Broder, S. (1984) *Science (Washington, D.C.) 226*, 172–174.
Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Nusinoff-Lehrman, S., Gallo, R. C., Bolognesi, D., Barry, D. W., & Broder, S. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7096–7100.
Nakajima, K., Ono, K., & Ito, Y. (1974) *Intervirology 3*, 324–341.
Nakane, H., Balzarini, J., De Clercq, E., & Ono, K. (1988) *Eur. J. Biochem. 177*, 91–96.
Okamura, S., Crane, F., Messner, H. A., & Mak, T. W. (1978) *J. Biol. Chem. 253*, 3765–3767.
Ono, K., Ohashi, A., Tanabe, K., Matsukage, A., Nishizawa, M., & Takahashi, T. (1979) *Nucleic Acids Res. 7*, 715–726.
Ono, K., Iwata, Y., & Nakane, H. (1983) *Biomed. Pharmacother. 37*, 27–35.
Ono, K., Nakane, H., Barrê-Sinoussi, F., & Chermann, J.-C. (1988a) *Eur. J. Biochem. 176*, 305–310.
Ono, K., Nakane, H., & Fukushima, M. (1988b) *Eur. J. Biochem. 172*, 349–353.
Ono, K., Nakane, H., Fukushima, M., Chermann, J.-C., & Barrê-Sinoussi, F. (1989a) *Biochem. Biophys. Res. Commun. 160*, 982–987.
Ono, K., Nakane, H., Fukushima, M., Chermann, J.-C., & Barrê-Sinoussi, F. (1989b) *Biomed. Pharmacother.* (in press).
Ono, K., Nakane, H., Meng, Z.-M., Ose, Y., Sakai, Y., & Mizuno, M. (1989c) *Chem. Pharm. Bull. 37*, 1810–1812.
Rozenbaum, W., Dormont, D., Spire, B., Vilmer, E., Gentilini, M., Griscelli, C., Montagnier, L., Barrê-Sinoussi, F., & Chermann, J.-C. (1985) *Lancet i*, 450–451.
Yamaguchi, M., Matsukage, A., & Takahashi, T. (1980) *J. Biol. Chem. 255*, 7002–7009.
Yarchoan, R., Klecker, R. W., Weinhold, K. J., Markham, P. D., Lyerly, H. K., Durack, D. T., Gelmann, E., Nusinoff-Lehrmann, S., Blun, R. M., Barry, D. W., Shearer, G. M., Fischl, M. A., Mitsuya, H., Gallo, R. C., Collins, J. M., Bolognesi, D. P., Myers, C. E., & Broder, S. (1986) *Lancet i*, 575–580.

Characterization of cDNAs of the Human Pregnancy-Specific β1-Glycoprotein Family, a New Subfamily of the Immunoglobulin Gene Superfamily†,‡

Qiao-Xi Zheng, Le Ann Tease, W. Lesley Shupert, and Wai-Yee Chan*
Departments of Pediatrics, Biochemistry, Anatomy, and Cell Biology, Georgetown University Medical Center, 3800 Reservoir Road, NW, Washington, D.C. 20007
Received August 11, 1989; Revised Manuscript Received November 2, 1989

ABSTRACT: Three highly homologous cDNAs encoding human pregnancy-specific β1-glycoprotein (SP1) were isolated from a human placental cDNA library. These cDNAs share >90% nucleotide homology in their coding sequences, and >79% of the encoded amino acids are homologous. Proteins encoded by these cDNAs are very similar to members of the carcinoembryonic antigen family and contain repeating domains, conserved disulfide bridges, and β-sheet structure typical of the immunoglobulin gene superfamily. However, the high degree of sequence homology and relatively lesser degree of glycosylation among the SP1 proteins suggest that they exist as a unique family instead of being members of the CEA family. Both soluble and potentially membrane-bound forms of SP1 proteins were present in the placenta. Northern blot analysis using specific probes confirmed the expression of multiple mRNA species in human term placenta.

Pregnancy-specific β1-glycoprotein (SP1) is an early pregnancy protein that can be detected in maternal serum as early as 16 days after conception (Grudzinskas et al., 1977). The maternal serum level of SP1 increases with progression of gestation and has been shown to be a good index for monitoring fetal growth and pregnancy complications (Bischof, 1984). SP1 is also elevated in serum of patients with tumors of trophoblastic origin as well as some nontrophoblastic tumors (Sorensen, 1982). The physiological function of SP1 is not known. Like many of the early pregnancy proteins, it has been suggested to be an immunosuppressive agent enhancing survival of the fetus (Cerni et al., 1977). Human placental SP1 as first reported (Bohn, 1972) is a single-subunit glycoprotein with a molecular mass of 90 kDa, 29% of which is carbohydrate. This protein, besides being shown to be synthesized by the syncytiotrophoblasts of the placenta (Horne et al., 1976), was recently shown to be also produced in extraplacental sites including intestine, testis, and uterus (Chan et al., 1988a–c). Further studies indicate that SP1 as it was initially defined might be composed of a group of highly homologous proteins (Chan et al., 1988a). These different species of SP1 proteins are the products of a family of genes. Several species of

† Supported in part by NIH Grant HD 21793 and by the Oklahoma Medical Research Foundation.
‡ The nucleotide sequences reported for hPS12 and hPS2 have been submitted to GenBank under Accession Numbers J02892 and J02893.
* Author to whom correspondence should be addressed at the Department of Pediatrics.

0006-2960/90/0429-2845$02.50/0