The Cytotoxicity of Cysteinylcatechols and Related Compounds to Human Melanoma Cells In Vitro

Ichiro Yamada, M.D., Sachiko Seki, M.D., Osamu Matsubara, M.D., Shosuke Ito, Ph.D., Soji Suzuki, M.D., and Tsutomu Kasuga, M.D.

Second Department of Pathology and Department of Radiology, School of Medicine, Tokyo Medical and Dental University (IY, SS, OM, SS, TK), Tokyo, and School of Hygiene, Fujita-Gakuen Health University (SI), Aichi, Japan

L-3,4-Dihydroxyphenylalanine (L-dopa) and its structural analogs are known to be potently cytotoxic to melanoma cells. We examined the effects of cysteinylcatechols and related compounds, which were newly synthesized as cysteinyl derivatives of L-dopa, on the growth of human melanoma cells in vitro, and their actions were compared with those of L-dopa. 4-S- and 3-S-Cysteinylcatechols showed significantly more potent cytotoxicity to melanoma cells than did L-dopa, and 2-S-cysteinylhydroquinone was next to the catechols in potency. The mechanism of action may involve interaction with the melanocyte-specific enzyme, tyrosinase, for which the cysteinylcatechols could become a better substrate than L-dopa itself. 4-S-Cysteaminylphenol was almost comparable to L-dopa in cytotoxicity, suggesting that this phenol might be oxidized to the corresponding catechol by tyrosinase within the melanoma cells. *J Invest Dermatol 88:538–540, 1987*

Polyphenolic intermediates in the formation of melanin from tyrosine are known to be potentially cytotoxic agents [1–3]. Wick et al showed that L-3,4-dihydroxyphenylalanine (L-dopa) is selectively toxic to melanoma cells in vitro [4] and that its chemical analogs also exhibit antitumor activity in experimental tumor systems [5–9]. Recently, Ito et al [10] found that 5-S-cysteinyl-3,4-dihydroxyphenylalanine (5-S-cysteinyldopa), an intermediate in the metabolic pathway from L-dopa to pheomelanin, is much more cytotoxic than L-dopa itself and suggested that the catechols with the cysteinyl group show a potent antitumor activity. In an attempt to obtain more effective agents, we have newly synthesized various cysteinyl derivatives as structural analogues of L-dopa and studied the effects of these drugs on the growth of cultured human malignant melanoma cells.

MATERIALS AND METHODS

Chemicals The 7 new drugs were synthesized by one of the authors (S.I.); the details of the chemical synthesis have been reported elsewhere [11]. The drugs could be classified into 2 groups on the basis of chemical structures; one was the diphenol group, which contained 2 hydroxyl groups as side chains. This group included 4-S-cysteinylcatechol (4-S-CC), 3-S-cysteinylcatechol.HCl.H_2O (3-S-CC), 3-S-cysteinyl-5-methylcatechol (3-S-C-5-MC), 2-S-cysteinylhydroquinone (2-S-CH), and 2-S-cysteinylresorcinol (2-S-CR). 4-S-Cysteinylcatechol had the chemical structure in which only a sulfur atom was introduced into a molecule of L-dopa. The other group was phenol, and it included 4-S-cysteaminylphenol (4-S-CAP) and 4-S-cysteinylphenol (4-S-CP). L-Dopa was purchased from Sigma Chemical Co. (St. Louis, Missouri). All the drug solutions were freshly prepared in Ham's F-10 medium (Gibco Laboratories, Grand Island, New York) just before use at the beginning of each experiment.

Cells The human malignant melanoma cell line (HMV-I) used in this study was established from a black-brown malignant melanoma in the vaginal wall of a 65-year-old woman [12]. The HMV-I cells were maintained in Ham's F-10 medium supplemented with 10% calf serum (Flow Laboratories Inc., Rockville, Maryland), penicillin (100 U/ml), and streptomycin (100 μg/ml), and incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Effects of the Drugs on Cell Growth Cells (1×10^5) were plated in 35-mm plastic dishes (tissue culture Petri dish; Falcon). After 48 h of incubation, the medium was replaced with fresh culture medium containing the desired concentrations of each drug. The range of drug concentrations was from 2 μg/ml to 2.2 mg/ml, although it varied depending on effects of the drugs. Duplicate cultures were set up at each of the concentrations. For each drug, assays were performed at least 3 times. After 48 h of drug exposure at 37°C, the medium was removed and the cells were trypsinized. The number of cells per dish, assessed by trypan blue exclusion, was determined microscopically with a hemocytometer. Under the experimental conditions provided, the average doubling time of control cells was 21.0 h.

The average number of cells in each treated culture was expressed as a percentage of the average number of cells in the control cultures without drugs. The IC_{50} value was defined as the

Manuscript received March 17, 1986; accepted for publication October 2, 1986.

This work was supported in part by Grants-in-Aid for Cancer Research (58010030, 59010027) and Scientific Research (58480154) from the Ministry of Education, Science and Culture of Japan.

Reprint requests to: Ichiro Yamada, M.D., Department of Radiology, School of Medicine, Tokyo Medical and Dental University, 5-45 Yushima 1-chome, Bunkyo-ku, Tokyo 113, Japan.

Abbreviations:

L-dopa: L-3,4-dihydroxyphenylalanine
IC_{50}: 50% growth inhibition concentration
2-S-CH: 2-S-cysteinylhydroquinone
2-S-CR: 2-S-cysteinylresorcinol
3-S-CC: 3-S-cysteinylcatechol
3-S-C-5-MC: 3-S-cysteinyl-5-methylcatechol
4-S-CAP: 4-S-cysteaminylphenol
4-S-CP: 4-S-cysteinylphenol
4-S-CC: 4-S-cysteinylcatechol

0022-202X/87/$03.50

dose effective in inhibiting 50% of the cell population growth after 48 h of exposure to the drug. The IC_{50} value was determined from a linear regression with the aid of a computer. The significance of difference of response to different drugs was based on Student's *t*-test.

RESULTS

Figure 1 shows the dose-response curve of each drug to HMV-I human melanoma cells after 48 h of drug exposure, and the IC_{50} value of each drug was summarized in Table I.

The IC_{50} value of 3-S-CC was 12.6 μg/ml and that of 4-S-CC, 15.7 μg/ml; the 2 cysteinylcatechols showed the most potent growth-inhibitory effect of the drugs examined in this study. The IC_{50} value of L-dopa examined for the purpose of comparison was 22.5 μg/ml, and 3-S-CC and 4-S-CC had a significantly potent effect on HMV-I cells compared with L-dopa ($p < 0.05$ for each drug).

2-S-Cysteinylhydroquinone had an IC_{50} value of 25.4 μg/ml. This was next to the 2 cysteinylcatechols in potency and was comparable to L-dopa ($p > 0.05$). However, the IC_{50} value of 3-S-C-5-MC was 29.4 μg/ml, about 2 times larger than that of 3-S-CC ($p < 0.05$), and this indicated that the introduction of a methyl group in the C-5 position of 3-S-CC resulted in a decrease of the effectiveness. 2-S-Cysteinylresorcinol had an IC_{50} value of 244.3 μg/ml and was the least effective of the diphenols.

With respect to the phenols, the IC_{50} value of 4-S-CAP was 24.1 μg/ml, and this phenol showed a growth-inhibitory effect comparable to L-dopa, a catecholic compound ($p > 0.05$). However, 4-S-CP had an IC_{50} value of 952.2 μg/ml and was the least effective.

Table I. Fifty Percent Growth Inhibition Concentration (IC_{50}) of the Drugs to HMV-I Human Melanoma Cells

Drug	IC_{50} (μg/ml)[a]	IC_{50} Ratio (L-dopa/drug)	Significance (*p* value)[b]
3-S-CC	12.6	1.79	< 0.05
4-S-CC	15.7	1.43	< 0.05
L-Dopa	22.5	1.00	—
4-S-CAP	24.1	0.93	> 0.05
2-S-CH	25.4	0.89	> 0.05
3-S-C-5-MC	29.4	0.77	< 0.05
2-S-CR	244.3	0.09	< 0.01
4-S-CP	952.2	0.02	< 0.01

[a]Each IC_{50} value is an average of values from 3–5 separate experiments.
[b]The significance was calculated between L-dopa and other drugs as described in *Materials and Methods*.

DISCUSSION

3-S-Cysteinylcatechol and 4-S-CC showed the most potent growth-inhibitory effect of the drugs examined in this study and were significantly more effective than L-dopa, another catechol. In general, 2 mechanisms have been postulated concerning the cytotoxicity of catechols [6,7,13]; one is that catechols are converted to the corresponding benzoquinones, and the quinones produce damage to the cells through inactivation of sulfhydryl enzymes, such as DNA polymerase α, which play a central role in the cell growth. The other mechanism is that the catechols are autooxidized to produce cytotoxic hydrogen peroxide, superoxide, and hydroxyl radicals. It is well known that, in melanoma cells, the conversion of L-dopa and its analogs to quinones can be mediated by the specific enzyme tyrosinase [14], and the quinones have been demonstrated to have a marked affinity for the sulfhydryl enzymes [2] and form covalent binding with the sulfhydryl groups [15].

The reason why the cysteinylcatechols are more cytotoxic to melanoma cells than L-dopa is yet to be studied. However, it could be ascribed to the presence of a sulfur atom in their molecule. The sulfur atom can increase lipophilic properties and pro-

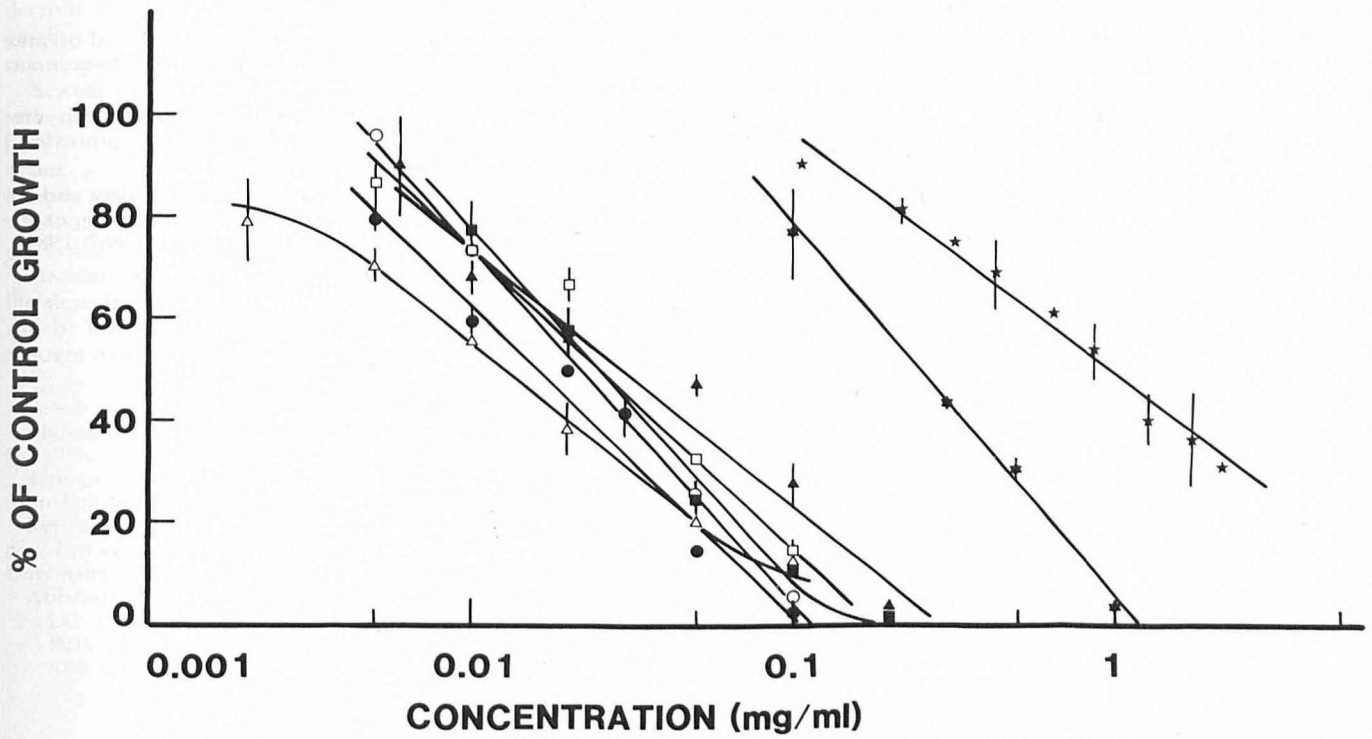

Figure 1. Effects of the drugs on the growth of HMV-I human melanoma cells. The cells were exposed to the different drug concentrations in the conditions described in *Materials and Methods*. The results were expressed as the percentage of control cell growth after 48 h of drug exposure. Values represent mean ± SE of 3–5 determinations. *Open triangles*, 3-S-CC; *closed circles*, 4-S-CC; *open circles*, L-dopa; *closed squares*, 4-S-CAP; *open squares*, 2-S-CH; *closed triangles*, 3-S-C-5-MC; *six-sided stars*, 2-S-CR; *five-sided stars*, 4-S-CP.

mote efficient uptake into the cells [11]. In addition, our ongoing study indicated that the cysteinylcatechols could become a better substrate for tyrosinase prepared from mushroom and mammalian melanoma cells than L-dopa. This may be due to the electron-donating resonance effect of the sulfur atom, which can increase the affinity for tyrosinase [16].

2-S-Cysteinylhydroquinone was second to the catechols in cytotoxicity. Hydroquinone itself has been known to have selective toxicity against melanocytes in vivo and is used clinically as a depigmenting agent [17,18]. Chavin et al [19] showed that hydroquinone significantly prolongs the survival of melanoma-bearing mice. Recently, Penney et al [20] suggested that the cytotoxicity of hydroquinone to melanoma cells may be via its oxidation by tyrosinase. Therefore, it is possible that the cytotoxicity of 2-S-CH to melanoma cells, comparable to L-dopa in potency, is also due to its tyrosinase-mediated oxidation. 2-S-Cysteinylresorcinol was the least effective among the diphenols, and this may be due to the fact that resorcinol is chemically unable to undergo oxidation to a quinone [21].

Our results demonstrate that 4-S-CAP, a phenol, is much more cytotoxic to melanoma cells than is 4-S-CP, another phenol, and 4-S-CAP is almost comparable to L-dopa, a catechol, in potency. The reason for this is not clear at present. However, removal of the carboxyl group from 4-S-CP can increase the lipophilic properties, and there may be efficient uptake into the cells [11]. It is possible that 4-S-CAP incorporated into melanoma cells is oxidized by tyrosinase to produce the corresponding catechol, which in turn exerts a potent cytotoxic effect comparable to L-dopa.

REFERENCES

1. Hochstein P, Cohen G: The cytotoxicity of melanin precursors. Ann NY Acad Sci 100:876–886, 1963
2. Graham DG, Tiffany SM, Vogel FS: The toxicity of melanin precursors. J Invest Dermatol 70:113–116, 1978
3. Pawelek JK, Lerner AB: 5,6-Dihydroxyindole is a melanin precursor showing potent cytotoxicity. Nature 276:627–628, 1978
4. Wick MM, Byers L, Frei III E: L-Dopa: selective toxicity for melanoma cells in vitro. Science 197:468–469, 1977
5. Wick MM: L-Dopa methyl ester as a new antitumor agent. Nature 269:512–513, 1977
6. Wick MM: L-Dopa methyl ester: prolongation of survival of neuroblastoma-bearing mice after treatment. Science 199:775–776, 1978
7. Wick MM: Dopamine: a novel antitumor agent against B-16 melanoma in vivo. J Invest Dermatol 71:163–164, 1978
8. Wick MM: Levodapa and dopamine analogs: melanin precursors as antitumor agents in experimental human and murine leukemia. Cancer Treat Rep 63:991–997, 1979
9. Wick MM, Byers L, Ratliff J: Selective toxicity of 6-hydroxydopa for melanoma cells. J Invest Dermatol 72:67–69, 1979
10. Fujita K, Ito S, Inoue S, Yamamoto Y, Takeuchi J, Shamoto M, Nagatsu T: Selective toxicity of 5-S-cysteinyldopa, a melanin precursor, to tumor cells in vitro and in vivo. Cancer Res 40:2543–2546, 1980
11. Ito S, Inoue S, Yamamoto Y, Fujita K: Synthesis and antitumor activities of cysteinyl-3,4-dihydroxyphenylalanines and related compounds. J Med Chem 24:673–677, 1981
12. Kasuga T, Furuse T, Takahashi I, Tsuchiya E: Ultrastructural and autoradiographic studies on melanin synthesis and membrane system using cultured B16 melanoma, irradiated melanoma, and human malignant melanoma, in Biology of Normal and Abnormal Melanocytes. Edited by T Kawamura, TB Fitzpatrick, M Seiji. Tokyo, Univ of Tokyo Press, 1971, pp 241–264
13. Graham DG, Tiffany SM, Bell WR Jr, Gutknecht WF: Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds towards C1300 neuroblastoma cells in vitro. Mol Pharmacol 14:644–653, 1978
14. Wick MM: Levodopa and dopamine analogs as DNA polymerase inhibitors and antitumor agents in human melanoma. Cancer Res 40:1414–1418, 1980
15. Kato T, Ito S, Fujita K: Tyrosinase-catalyzed binding of 3,4-dihydroxyphenylalanine with proteins through the sulfhydryl group. Biochim Biophys Acta 881:415–421, 1986
16. Duckworth HW, Coleman JE: Physicochemical and kinetic properties of mushroom tyrosinase. J Biol Chem 245:1613–1625, 1970
17. Bleehen SS, Pathak MA, Hori Y, Fitzpatrick TB: Depigmentation of skin with 4-isopropylcatechol, mercaptoamine, and other compounds. J Invest Dermatol 50:103–117, 1968
18. Jimbow K, Obata H, Pathak MA, Fitzpatrick TB: Mechanism of depigmentation by hydroquinone. J Invest Dermatol 62:436–449, 1974
19. Chavin W, Jelonek EJ Jr, Reed AH, Binder LR: Survival of mice receiving melanoma transplants is promoted by hydroquinone. Science 208:408–410, 1980
20. Penney KB, Smith CJ, Allen JC: Depigmenting action of hydroquinone depends on disruption of fundamental cell processes. J Invest Dermatol 82:308–310, 1984
21. Wick MM: Levodopa and dopamine analogs: dihydroxy and trihydroxybenzylamines as novel quinol antitumor agents in experimental leukemia in vivo. Cancer Treat Rep 65:861–867, 1981