Selective Toxicity of 5-S-Cysteinyldopa, a Melanin Precursor, to Tumor Cells in Vitro and in Vivo

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ABSTRACT

The effect of 5-S-cysteinyldopa, an intermediate in the pathway from L-3,4-dihydroxyphenylalanine (l-dopa) to melanin, on the growth of eight human tumor cell lines in culture was compared to that of l-dopa. The tumor cell lines tested comprised two neuroblastomas (NB-1 and YT-nu), two amelanotic melanomas (HMV and SEKI), a gastric carcinoma (MKN-28), and three squamous cell carcinomas (HeLa-S3, KB, and a salivary gland carcinoma). Cys-dopa at a concentration of 1 mm inhibited growth of NB-1 (66%), YT-nu (67%), HMV (44%), SEKI (60%), MKN-28 (47%), HeLa-S3 (24%), KB (64%), and salivary gland carcinoma (33%), while l-dopa exhibited similar or even lower degrees of inhibition at a concentration of 6 mm. On the other hand, both catechols had little effect on the growth of two fibroblasts derived originally from normal tissues (mouse fibroblast L929 and Chinese hamster fibroblast Don-6). Cys-dopa and l-dopa inhibited DNA and protein synthesis in YT-nu cells, but RNA synthesis was less affected. Treatment with cys-dopa at a dose of 1000 mg/kg i.p. for 7 days prolonged by 50% the life span of mice inoculated with L1210 leukemia. Normal mice given cys-dopa at a dose of 1000 mg/kg for 12 days showed no signs of toxicity. These results suggest the potential of cys-dopa as an antitumor agent.

INTRODUCTION

Cys-dopa is an intermediate in the metabolic pathway from l-dopa to the red-brown pigment, melanin. This unique amino acid was newly detected in human melanoma (1) and in the urine of melanoma patients (1), and it was suggested that determination of this thiocatechol in the urine may be of practical clinical value (1). Recently, Wick et al. (23) reported that L-dopa has a selective toxicity for melanotic melanoma cells in vitro. L-Dopa methyl ester, a more soluble derivative of L-dopa, had in vivo antitumor activity against murine melanoma, leukemias, and neuroblastoma (19, 21), and dopamine was also effective against B-16 melanoma in vivo (20). These reports prompted us to examine antitumor activity of cys-dopa, another immediate metabolite of L-dopa. We studied the effect of cys-dopa on the growth of 8 human tumor lines, including 2 neuroblastomas and 2 amelanotic melanomas, in comparison with L-dopa. In vitro antitumor activity of cys-dopa against murine L1210 leukemia and B-16 melanoma is also reported.

MATERIALS AND METHODS

Chemicals. Cys-dopa was prepared by a chemical method (15) and was separated from 2-S-cysteinyldopa and other products by Dowex column chromatography (8). The amino acid was crystallized in the free form and gave correct elemental and analytical data for C12H13N2O5·H2O (M.W. = 334). L-Dopa was purchased from Sigma Chemical Co., St. Louis, Mo., and [1H]thymidine (specific activity, 2 Ci/mmol), [5-3H]uridine (specific activity, 28 Ci/mmol), and [L-3H]leucine (specific activity, 40 to 60 Ci/mmol) were from New England Nuclear, Boston, Mass.

Cell Lines. The cell line HMV (10), derived from a malignant melanoma of the vagina in a 65-year-old Japanese woman, was provided by Professor T. Kasuga (Department of Pathology, School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan), and the cell line SEKI, established from a malignant melanoma of the skin in a 28-year-old Japanese woman, was given by Professor J. Sato (Department of Pathology, School of Medicine, Okayama University, Okayama, Japan). Both HMV and SEKI cells had melanin-synthesizing activity during the early transfer generations, but the melanomas could not be observed in these cells at the present generation in our laboratory. However, premelanomas were sparsely found in SEKI cells. Melanotic melanoma cell lines were not available for this study. The cell lines of human neuroblastoma NB-1 and YT-nu (7) and that of human gastric carcinoma MKN-28, derived from a well-differentiated tubular adenocarcinoma in a 70-year-old Japanese woman (6), were gifts from Professor T. Suzuki (Department of Pathology, School of Medicine, Niigata University, Niigata, Japan). Human squamous cell carcinomas, HeLa-S3 and KB, mouse fibroblast L929, and Chinese hamster fibroblast Don-6 were gifts from Aichi Cancer Center Research Institute, Nagoya, Japan. A cell line of squamous cell carcinoma of the salivary gland was established in our laboratory (24). The carcinoma cells had a lot of tonofilaments and desmosomes in vitro. These cell lines have been maintained in McCoy’s 5A medium (Microbiological Associates), 10% fetal bovine serum (Microbiological Associates), and 100 units of penicillin per ml (Meiji Seika Co., Tokyo, Japan), and 100 μg of streptomycin per ml (Meiji Seika Co.).

Effects of Cys-dopa and L-Dopa on Cell Growth. The meth-
For this experiment were essentially similar to those described by Wick et al. (23). Single cell suspensions in McCoy's 5A medium or Eagle's minimum essential medium were inoculated into 60-mm Falcon Petri dishes, and cells were allowed to attach for 24 hr prior to exposure to cys-dopa or L-dopa. After the cells were washed, 1 ml of HBSS containing cys-dopa or L-dopa was added, and cultures were incubated at 37° for 1 hr. The exposed cells were then grown in McCoy's 5A medium or Eagle's minimum essential medium for 48 hr. Cells were harvested by trypsinization with 0.25% trypsin-EDTA and counted in a Model Z Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). Under the experimental conditions, control cells had the following doubling times: NB-1, 66 hr; YT-nu, 58 hr; HMV, 24 hr; SEKI, 30 hr; MKN-28, 36 hr; Hepa-S3, 30 hr; KB, 48 hr; salivary gland carcinoma, 36 hr; L929, 24 hr; and Don-6, 25 hr.

Effects of Cys-dopa and L-Dopa on Macromolecule Biosynthesis. The methods for this experiment were also similar to those described by Wick (21), except that the exposure to drug and labeled precursor was carried out in HBSS instead of McCoy's 5A medium. This change was made in order to compare the effects of cys-dopa and L-dopa on macromolecule biosynthesis under the same conditions as those for the growth inhibition experiments. The YT-nu neuroblastoma cells were grown in McCoy's 5A medium and plated in Linbro multiwell tissue culture trays. After the cells had been plated for 24 hr, they were washed with 0.9% NaCl solution, and 1 ml of HBSS containing 2 μCi of [3H]thymidine, [3H]uridine, or [3H]leucine, and cys-dopa or L-dopa was added. After incubation for 60 min at 37°, the HBSS was removed, cells were washed once with 0.9% NaCl solution, and 1 ml of 10% trichloroacetic acid was added. After 30 min at room temperature, the precipitate was washed 3 times with 0.9% NaCl solution, and 0.5 ml of 1 M KOH was added. After digestion at 37° for 4 hr, an aliquot was added to scintillation fluid (Aquasol-2; New England Nuclear) and counted in a Packard 2650 scintillation counter.

Effects of Cys-dopa on Survival of L1210- and B-16-bearing Mice. L1210 leukemia and B-16 melanoma have been maintained by following the National Cancer Institute protocols (4). On Day 0, young male C57BL/6 × DBA/2 F1 (hereafter called BDF) mice, 18 to 20 g, were given i.p. inoculations of either 1 × 10⁵ L1210 leukemia cells or 5 × 10⁶ B-16 melanoma cells. Treatment of mice, 10 animals/group, was begun on Day 1 and continued daily for 7 days to L1210-bearing mice and for 12 days to B-16-bearing mice. Cys-dopa, dissolved in 1 ml of 0.9% NaCl solution, was given i.p. once a day. Control animals received i.p. injections of 1 ml of 0.9% NaCl solution.

Toxicity of Cys-dopa in Mice. A solution of cys-dopa in 0.9% NaCl solution was injected i.p. into 6 male BDF mice at a dose of 1000 mg/kg for 12 consecutive days. The mice were observed for signs of toxicity and weighed regularly. Of 6 mice, 3 were sacrificed 1 day after the termination of injections, and selected tissues were excised, fixed in formalin, embedded in paraffin, and sectioned. The tissue sections were stained with hematoxylin-eosin and periodic acid-Schiff reagent.

RESULTS

Effects of Cys-dopa and L-Dopa on Cell Growth. Chart 1 shows the dose-response curves for growth inhibition of human tumor cell lines 48 hr after treatment with cys-dopa or L-dopa for 1 hr in HBSS. Cys-dopa at a concentration of 1 mM inhibited the growth of neuroblastomas (NB-1 and YT-nu), amelanotic melanomas (HMV and SEKI), a gastric carcinoma (MKN-28), and a squamous cell carcinoma (KB) by more than 40%. The growth of the other 2 cell lines, Hepa-S3 and a salivary gland carcinoma, was inhibited to a lesser extent. L-Dopa inhibited the growth of these tumor cells as well, but at much higher concentrations. Cell lines HMV and Hepa-S3 were least affected by L-dopa. Two cell lines derived originally from normal tissues, mouse fibroblast L929 and Chinese hamster fibroblast Don-6, were essentially unaffected by both cys-dopa and L-dopa. Thus, cys-dopa had a selective toxicity for tumor cells and, on the average, is approximately 10 times more potent than L-dopa. Exposure for up to 48 hr gave similar results with lower concentrations of cys-dopa, but L-dopa lost selectivity owing to autoxidation.

Effects of Cys-dopa and L-Dopa on Macromolecule Biosynthesis. Table 1 summarizes the effects of cys-dopa and L-dopa on thymidine, uridine, and leucine incorporation in YT-nu neuroblastoma cells. Both catechols exhibited a similar pattern of inhibition with greater effects on DNA and protein synthesis. The effect of cys-dopa at a concentration of 0.5 mM was comparable to that of L-dopa at a concentration of 1.5 mM.
cantly prolonged the life span of mice bearing L1210 leukemia (Table 2). It was also slightly active against B-16 melanoma. Indications of toxicity such as early deaths or weight losses were not observed in the treated animals.

**Toxicity of Cys-dopa in Mice.** The acute i.p. dose of cys-dopa lethal to 50% of mice could not be estimated because of its low solubility. However, mice given cys-dopa i.p. at a dose of 1000 mg/kg for 12 consecutive days showed no signs of systemic toxicity as judged by their weight and activity. The test animals survived beyond 60 days after injections. Microscopic examinations disclosed no alterations in cells of livers, kidneys, and spleens that were excised 1 day after the administration of cys-dopa for 12 days. These results indicate a reduced in vivo toxicity of cys-dopa, compared with L-dopa, for which the acute i.p. dose lethal to 50% of mice is reported to be 1189 mg/kg (985 to 1390 mg/kg) (11).

**DISCUSSION**

Cys-dopa is toxic to a variety of human tumor cells in vitro and is approximately 10 times more potent than L-dopa. The amino acid is also active against L1210 leukemia and B-16 melanoma in vivo, with no untoward effects on the host. This is in contrast to L-dopa methyl ester and dopamine, which are neurotoxic and lethal at doses greater than 600 mg/kg, although they exhibited as good antitumor activities as cys-dopa (19–21). One possible mechanism for the reduced toxicity of cys-dopa might be related to its inability to be transformed to a neurotransmitter (22).

The mechanism of antitumor action of cys-dopa is yet to be studied. However, the results described here seem to indicate that cys-dopa and L-dopa function with similar mechanisms. Since both amino acids possess the same unique moiety, catechol, which is one of the most readily oxidizable groups in nature, the possible mechanism of action may involve oxidative activation of the catechol moiety. Wick (20, 21) postulated that catechol, which is one of the most readily oxidizable groups in cysteine molecule to form a diadduct, 2,5-S,S-dicysteinyldopa, to its ability to combine with sulfhydryl compounds. It is known that 5-S-cysteinyldopaquinone is able to combine with another cysteine molecule to form a diadduct, 2,5-S,S-dicysteinyldopa (8). The enzymes capable of the conversion of catechols to 1,2-benzoquinones are tyrosinase, which is localized in melanocytes, and peroxidase (2), the activity of which has been demonstrated in a variety of cells (13). The same type of oxidation can also be promoted by superoxide radical, the level of which may be higher in tumor cells than in normal cells in consequence of the lowered level of superoxide dismutase (12). Scheulen et al. (17) and Dybing et al. (3) reported evidence indicating that L-dopa can bind covalently with sulfhydryl groups by the action of xanthine oxidase-generated superoxide radical. Since not only cys-dopa but also L-dopa inhibits growth of various human tumor cells which lack tyrosinase activity, enzyme systems other than tyrosinase should be responsible for the oxidation of the catechols in these tumor cells. However, it may be possible that the antitumor activities of the catechols against melanotic B-16 melanoma are mediated mainly by tyrosinase. The rather poor chemotherapeutic effect of cys-dopa against B-16 melanoma could be related to the fact that it is a much poorer substrate for tyrosinase than is L-dopa (9). We are currently investigating whether cys-dopa as well as L-dopa can bind with cysteine by the action of peroxidase-hydrogen peroxide or enzymically generated superoxide radical.

**REFERENCES**


**Table 2**

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<th>Tumor</th>
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<th>Survival time (days)</th>
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<td>500</td>
<td>11</td>
<td>9–18</td>
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<td>12</td>
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<td>21–33</td>
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</table>

%ILS, percentage of increase in life span, of treated versus control animals.

* Significant at p < 0.001.

c Significant at p < 0.05.


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