Antimelanoma Effect of 4-S-Cysteaminylcatechol, an Activated Form of 4-S-Cysteaminylphenol

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ABSTRACT

Rational chemotherapy of malignant melanoma could be developed by taking advantage of the presence of melanogenic enzymes in melanoma cells. 4-S-Cysteaminylphenol (4-S-CAP) has been evaluated for melanocytotoxicity and antimelanoma effect. Although 4-S-CAP is selectively toxic to pigmented melanoma cells, it is not potent enough when applied as a single agent. To increase the efficacy of 4-S-CAP, we synthesized 4-S-cysteaminylcatechol (4-S-CAC), an activated form of 4-S-CAP, and compared its biochemical properties and antimelanoma effects with those of the isomers 3-S-cysteaminylcatechol (3-S-CAC) and 2-S-cysteaminylhydroquinone (2-S-CAH). 4-S-CAC was found to be a better substrate for melanoma tyrosinase than was 1,3,4-dihydroxyphenylalanine, the natural catechol substrates. 3-S-CAC was a poor substrate, whereas 2-S-CAH was not a substrate. 4-S-CAC was the most cytotoxic to three lines of melanoma cells in vitro, followed by 2-S-CAH and 3-S-CAC. When applied i.p. for 9 days at a dose of 100 mg/kg, 4-S-CAC-HCl, increased by 46−52% the life span of C57BL/6 mice inoculated i.p. with B16 melanoma; this effect was comparable to that of a 50 mg/kg dose of 5-(3,3-dimethyl-triazentyl)-1H-imidazole-4-carboxamide. 3-S-CAC was marginally effective, whereas 2-S-CAH was toxic to the host. This systemic toxicity of 2-5-CAH, to which requests for susceptibility to autooxidation. Growth of B16 melanoma cells inoculated s.c. was significantly inhibited by i.p. administration of 4-S-CAC-HCl (200 mg/kg) for 5 days (P<0.05). These results suggest that 4-S-CAC is a potent antimelanoma agent, the effect of which is mostly mediated through tyrosinase oxidation.

INTRODUCTION

Melanogenesis is a biochemical property unique to melanoma cells among cancers. In normal and malignant cells, the specific enzyme tyrosinase catalyzes the oxidative conversion of the common amino acid L-tyrosine to melanin pigments (reviewed in Ref. 1). The first steps are the hydroxylation of L-tyrosine to form L-dopa and the subsequent oxidation of L-dopa to L-dopaquinone. L-Dopaquinone, an o-quinone, is highly reactive and immediately undergoes cyclization followed by oxidation to form a more stable intermediate, L-dopachrome. The rearrangement of dopachrome leads to 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. These dihydroxyindoles are oxidized to form indolequinones, which are polymerized to form ultimately the brown-black pigment, eumelanin. It has recently been shown that TRP1, another enzyme unique to pigmented cells, also catalyzes oxidation of 5,6-dihydroxyindole-2-carboxylic acid to the indolequinone, whereas tyrosinase oxidizes 5,6-dihydroxyindole but not its carboxylic acid (2).

Hochstein and Cohen(3) suggested as early as 1963 that reactive quinoid intermediates generated during melanogenesis could be toxic to the cell in which they are produced, i.e., the melanocyte. In 1969, Riley (4, 5) introduced 4-methoxyphenol (4-hydroxyanisole) as a melanocytotoxic compound. Subsequently, Wick, Byers, and Frei(6) and Wick (7) showed that L-dopa and related catechols (o-diphenols) are selectively toxic to melanoma cells. Since then, many groups have attempted to develop chemotherapeutic agents that exhibit selective toxicity to melanoma cells through oxidative conversion to quinoid intermediates (reviewed in Refs. 8 and 9). We have synthesized a number of phenolic and catecholic melanin precursors and evaluated their cytotoxicity in vitro and in vivo (10−12). Among the new compounds tested, 4-S-CAP appeared to be the most potentially promising antimelanoma agent (Fig. 1). 4-S-CAP is a good substrate for tyrosinase, and the oxidized form binds to thiol enzymes (13). In vitro experiments have shown that it is incorporated and becomes cytotoxic to melanoma cells (14, 15) and that it inhibits DNA synthesis in melanoma cells (16); these phenomena are correlated to the degree of pigmentation. The inhibition of DNA synthesis is correlated to inhibition of thymidylate synthase, a thiol enzyme essential for DNA synthesis and, eventually, cell survival (17). Furthermore, several in vivo studies have shown that 4-S-CAP accumulates in the eye and the tumor of a B16 melanoma-bearing mouse (14), inhibits growth of B16 mouse melanoma (12, 16, 18) and a human melanoma xenograft (19), and increases the life span of B16 melanoma-bearing mice (16, 18). The phenol also destroys melanocytes in hair follicles of black mice and reduces the number of B16F10 colonies in lungs (20).

However, 4-S-CAP is not potent enough to pursue further chemotherapeutic trials. Reasons for this limitation may be related to the strong hypotensive effect of 4-S-CAP (21) and its conversion to the aldehyde form through oxidation by plasma MAO (22); these properties result in a low LD₅₀ of 430 mg/kg (16). To increase the efficacy of 4-S-CAP, we newly synthesized 4-S-CAC, the catechol derivative of 4-S-CAP, and evaluated its biochemical properties and antimelanoma effects in vitro and in vivo. These properties were compared with those of 3-S-CAC and 2-S-CAH, the two isomers of 4-S-CAC. It is well known that the oxidation of L-dopa, a catechol, proceeds approximately two orders of magnitude faster than the hydroxylation of L-tyrosine, a phenol (23). Therefore, it was expected that, in melanoma cells, the oxidation of 4-S-CAC would proceed much faster than the hydroxylation (and hence oxidation) of 4-S-CAP, and thus, 4-S-CAC may exhibit a much higher antimelanoma effect than 4-S-CAP.

MATERIALS AND METHODS

Chemicals

4-S-CAP was prepared as described by Padgett et al. (21). L-dopa, DTIC, MBTH, and mushroom tyrosinase (5600 units/mg) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade. Melanoma tyrosinase and TRP1 were obtained from B16 mouse melanomas. These enzymes were purified according to the procedure published elsewhere (2, 24). The purity of the preparations was checked by SDS-PAGE and subsequent Western blots using specific antibodies αPEP1 (anti-TRP1) and...
cases.

catechol ("diphenol) derivative of 4-S-CAP and that 3-S-CAC and 2-S-CAH are the two possible isomers of 4-S-CAC.

HPLC

The HPLC system consisted of a Jasco PU-980 intelligent liquid chromatograph with a Jasco 851-AS intelligent sampler (JASCO, Tokyo, Japan), a Jasco 840-EC electrochemical detector, and a 4.6 x 150 mm (internal diameter) C18 reverse-phase column packed with Jasco Catecholpack (particle size, 7 μm). The mobile phase was 0.1 M potassium phosphate buffer (pH 2.1) containing 1 mM EDTA:methanol, 92:8 (v/v). The column was maintained at 30°C, and the flow rate was 0.7 ml/min. The diphenols were detected at 500 mV versus a mercury/mercury chloride reference electrode.

Melanoma Cell Lines

The origin of human melanoma cell lines HMV-II (pigmented) and HMV-I (nonpigmented) was described previously (22). These cells were cultured as monolayers at 37°C in Ham's F-10 medium (with glutamine; from GIBCO BRL, Grand Island, NY) supplemented with 15% FCS, (100 units/ml) penicillin, and (100 /ng/ml) streptomycin in a 5% CO2 atmosphere. The human melanoma cell line MM418 (highly pigmented) was obtained from Dr. P. G. Parsons (Queensland Institute of Medical Research, Brisbane, Australia). The cells were cultured in RPMI 1640 (GIBCO BRL) supplemented with 5% FCS, penicillin, and streptomycin.

Synthesis of Cysteaminyldiphenols

4-S-CAC-HCl and 2-S-CAH-HCl were prepared and purified in an essentially similar manner as that described for the preparation of 4-S-cysteinylocatechol (11). 3-S-CAC-HCl was prepared by the addition reaction of cysteamine to o-benzoquinone (11).

4-S-CAC-HCl. A mixture of 33 g (300 mmol) of pyrocatechol and 16.9 g (75 mmol) of cystamine dihydrochloride in 500 ml of 47% HBr was refluxed for 2 h. The reddish brown solution was evaporated to dryness in a rotary evaporator. The residue was dissolved in 100 ml of 3 M HC1 and extracted with ethyl acetate (3 x 100 ml) to remove byproducts. The aqueous layer was evaporated, and the residue was chromatographed on a Dowex 50W-X2 column (3.6 x 15 cm; equilibrated with 3 M HC1). The column was eluted with 3 M HC1, and fractions of 20 ml were collected and analyzed for 3-S-CAC and 2-S-CAH. The 4-S isomer was the major one. A concentrated solution of the mixture in ethanol was left in a freezer. Colorless needles of 4-S-CAC-HCl were precipitated and filtered and washed with acetone. 4-S-CAC and 3-S-CAC; the 4-S isomer was the major one. A concentrated solution of methanol containing 1 ml of 98% formic acid, were added 6 g of anhydrous sodium sulfate and 4.7 g (20 ml) of silver oxide at room temperature. The mixture was vigorously stirred for 5 min and filtered through a layer of sodium sulfate, and the filtered mass was washed with methanol. The combined, wine-red filtrate was added dropwise over 10 min to a stirred solution of 1.13 g (10 mmol) of cysteamine hydrochloride in 20 ml of water and 20 ml of methanol at room temperature. Five ml of 6% sulfuric acid were added to the solution, and the mixture was concentrated to approximately 25 ml. The residue was extracted with ethyl acetate (3 x 60 ml) to remove byproducts. The aqueous layer was evaporated, and the residue was chromatographed on a Dowex 50W-X2 column (3.6 x 10 cm). Elution with 3 M HC1 and evaporation of fractions containing 3-S-CAC left the HC1 salt of 3-S-CAC. Crystallization from ethanol yielded 1.45 g (65.4%) of 3-S-CAC-HCl as colorless needles: m.p. 141-144°C (dec.); UV (0.1 M HC1) Amax 265 nm (ε 5600) and 310 nm (3200). 1H and 13C NMR spectra were consistent with the structure of 3-S-CAC.

Biochemical Studies

Tyrosinase Oxidation and Autoxidation of Cysteaminyldiphenols. Reaction mixtures contained 0.1 mM 4-S-CAC, 3-S-CAC, or 2-S-CAH in 1 ml of 50 mM sodium phosphate buffer (pH 6.8). For tyrosinase oxidation, 5 μg of mushroom tyrosinase were added, and the oxidation was carried out at 37°C. At 5, 10, 20, and 30 min, a 10-μl aliquot of the reaction mixture was removed and mixed with 90 μl of 0.4 M HCIO4. The concentrations of the substrate remaining were measured by HPLC (see above). Autoxidation was carried out similarly but without tyrosinase. The results were averages for two separate experiments, with each experiment giving a similar result.

 Determination of Kinetic Constants for Melanoma Tyrosinase and TRP1. Reaction mixtures (1 ml total volume) were prepared by adding different concentrations of a substrate ranging from 0.2 to 1 mM in media consisting of 10 mM sodium phosphate buffer (pH 6.8), 0.1 mM EDTA, and 3 mM MBTH. After the addition of 2 milliunits (10 μg) of mouse melanoma tyrosinase or 1.4 milliunits (126 μg) of TRP1, the reactions were followed spectrophotometrically at 500 nm and 37°C, and the formation of the conjugates between MBTH and o-quinones formed from 4-S-CAC or L-dopa was recorded (25). The solution of 4-S-CAC was freshly prepared in each experiment to avoid autoxidation. The reaction was followed for 5 min against appropriate controls containing the same chemicals but lacking the enzyme. The Km and Vmax determinations were repeated twice and had good reproducibility.

In Vitro Cytotoxicity Assay

Exponentially growing melanoma cells were harvested, inoculated at a concentration of 2 x 10⁵ cells/dish into 60-mm Falcon Petri dishes, and allowed to attach for 24 h prior to exposure to the cysteaminyldiphenols. The cells were incubated continuously with either a regular medium or a medium containing one of the chemicals at concentrations of 10, 30, 100, and 300 mM. After 48 h of incubation, cells were harvested by trypsinization with 0.25% trypsin-EDTA and counted in a Sysmex F-610 Micro Cell Counter (Kobe, Japan). The IC50 were calculated on the basis of three separate experiments, with each experiment being performed in duplicate. In addition, cytotoxicity of 4-S-CAC was assayed in a serum-free medium. The experiments were performed in a similar manner as described above, but BIO-RICH 1 serum-free medium (Flow, McLean, VA) was used.

In Vivo Cytotoxicity Assay

Pigmented B16 melanoma has been maintained by following the National Cancer Institute protocol (26). On day 0, 5-week-old C57BL/6 x DBA/2 F₁
B16 melanoma cells (1 x 10^6 cells) were inoculated s.c. at the axillary region of 25 C57BL/6 mice (5-week-old mice; 18 - 20 g), and treatment was initiated on day 8, when tumors reached an estimated volume of approximately 100 mm^3. The 25 mice were divided into 5 groups of 5 mice each: control; 300 mg/kg 4-S-CAP; 100 mg/kg 4-S-CAC-HCl; 200 mg/kg 4-S-CAC-HCl; and 50 mg/kg DTIC. The chemicals, dissolved in 1 ml of 0.9% NaCl, were given i.p. once a day for 5 consecutive days. Control animals received 0.9% NaCl. Tumor diameters were measured with calipers on alternate days, and tumor volumes were calculated by the formula: long axis x (short axis)^2 x 1/2. Growth rates were expressed in log Vt/Vo, where Vt is the tumor volume at each measurement, and Vo is the tumor volume on day 8.

**RESULTS**

**Synthesis of Cysteaminyldiphenols.** The three isomers of cysteaminyldiphenols, 4-S-CAC, 3-S-CAC, and 2-S-CAH, were newly synthesized using standard procedures. They were prepared in gram scale, and 4-S-CAC and 3-S-CAC were obtained as crystals.

**Cysteaminyldiphenols as Substrates for Tyrosinase.** The cysteaminyldiphenols were examined as substrates for tyrosinase and for autoxidation. Fig. 2 shows that 4-S-CAC was rapidly oxidized by mushroom tyrosinase, whereas 3-S-CAC was oxidized very slowly. Although detailed kinetic experiments were not performed, 4-S-CAC was oxidized at least 5-fold faster than 3-S-CAC. 2-S-CAH did not act as a substrate for tyrosinase, as expected from the /_/diphenol structure. By autoxidation, 2-S-CAH was oxidized most rapidly, followed by 4-S-CAC and 3-S-CAC; the difference between the latter two was small.

Because 4-S-CAC was found to be a good substrate for mushroom tyrosinase, we next examined the efficacy of this catechol, in comparison with L-dopa, as a substrate for tyrosinase and TRP1 isolated from B16 mouse melanoma (2, 24). Oxidation of the catechols by these melanogenic enzymes was monitored spectrophotometrically at 500 nm following the formation of dark pink conjugates between MBTH and o-quinones (25). 4-S-CAC had a lower Km value and a higher Vmax value than L-dopa, the catecholic natural substrate for TRP1. Inhibition of TRP1 activity was not observed, even at 1 mM concentration of 4-S-CAC.

**In Vitro Cytotoxicity of Cysteaminyldiphenols.** The in vitro cytotoxicity of cysteaminyldiphenols was assessed against three lines of melanoma cells, a highly pigmented MM418, a moderately pigmented HMV-II, and a nonpigmented HMV-I (Table 2). The cysteaminyldiphenols were highly toxic to the melanoma cells; the IC_{50} values were in the 10 - 100 /μM range. 4-S-CAC was the most toxic, followed by 2-S-CAH and 3-S-CAC. The toxicity of 4-S-CAC was not affected by the difference in ability to synthesize melanin.

We have shown previously that in vitro cytotoxicity of 4-S-CAP was mostly mediated through its conversion to the aldehyde formed by the action of plasma MAO present in the FCS (22). Therefore, we examined the cytotoxicity of 4-S-CAC in a serum-free medium. The results, shown in Table 1, indicate that the cytotoxicity did not diminish, but rather increased, in the serum-free medium.

**In Vivo Cytotoxicity of Cysteaminyldiphenols.** 4-S-CAC-HCl was found to be highly cytotoxic to B16 melanoma cells inoculated i.p. to BDF1 mice, with the %ILS values being 46 and 49% at doses of 100 and 200 mg/kg, respectively (Table 3, Experiment 1). The effects were comparable to a 50-mg/kg dose of DTIC, which is the single most effective chemotherapeutic agent clinically used against malignant melanoma (27). The catechol was not toxic to the host, as judged by the body weight change. The two isomers of 4-S-CAC, i.e., 3-S-CAC and 2-S-CAH, were not effective against B16 melanoma;
3-S-CAC was marginally effective, whereas 2-S-CAH was toxic to the host. Because 4-S-CAC-HCl was found to be effective against B16 melanoma, we then compared its antimelanoma potential with 4-S-CAP. The results show that the catechol 4-S-CAC was much more effective in prolonging the life span of B16 melanoma-bearing mice than was the parent phenol 4-S-CAP (Table 3, Experiment 4).

**In Vivo Growth Inhibition of Melanoma.** We examined the efficacy of 4-S-CAC in inhibiting the growth of B16 melanoma inoculated s.c. to C57BL/6 mice (Fig. 3). At 14 days after tumor inoculation, the growth was inhibited by 67% in mice treated with a 200 mg/kg dose of 4-S-CAC-HCl (P < 0.05); this effect was slightly lower than that exhibited by a 50 mg/kg dose of DTIC. The antimelanoma effect of 4-S-CAP at a dose of 300 mg/kg was comparable to that of 4-S-CAC-HCl at a dose of 100 mg/kg, although their effects were not statistically significant.

**DISCUSSION**

In this study, we have shown that 4-S-CAC is a better substrate for tyrosinase than is L-dopa, and the two isomers are either a poor substrate or not a substrate at all. TRP1, a second oxidase unique to melanocytes, can oxidize 4-S-CAC as well, although at a much slower rate. 2-S-CAH was the most susceptible to autoxidation. These biochemical properties fit well with the in vivo cytotoxicity of the cysteaminyldiphenols to B16 mouse melanoma. 4-S-CAC is as potent as DTIC in increasing the life span of B16 melanoma-bearing mice, whereas 3-S-CAC is marginally effective, and 2-S-CAH is toxic to the host. The strong melanocytotoxicity of 4-S-CAC in *vitro* appears to correspond to its ability to be oxidized by tyrosinase and TRP1. These results suggest that the antimelanoma effect of 4-S-CAC is dependent on tyrosinase activity, and the systemic toxicity of 2-S-CAH is related to its susceptibility to autoxidation.

It is argued that catechols may not be suitable for antimelanoma agents in view of their susceptibility to autoxidation and systemic toxicity. However, mice tolerated a single i.p. administration of 400–600 mg/kg 4-S-CAC-HCl, and repeated administration at a 200 mg/kg dose did not give signs of systemic toxicity. In addition, we did not observe depigmentation of growing hair, such as was frequently seen when 4-S-CAP was administered to mice (12, 20).

A major limitation of 4-S-CAP is the strong hypotensive effect (21). It appears that this hypotensive effect of 4-S-CAP was abolished by its conversion to the catecholic structure of 4-S-CAC. MAO plays a major role in promoting the cytotoxicity of 4-S-CAP in *vitro* through its conversion to the aldehyde and the formation of H₂O₂ (22, 28). However, MAO does not seem to play such a role in 4-S-CAC, because 4-S-CAC exhibited a higher cytotoxicity in a serum-free medium than in a serum-containing medium (Table 2). It seems that the oxidative metabolism proceeds much faster than the MAO-dependent degradation.

Although 4-S-CAC is as potent as DTIC when applied i.p. to i.p.-transplanted B16 melanoma, the catechol is not as potent as DTIC when applied i.p. to the same melanoma transplanted s.c. This suggests that some of the 4-S-CAC administered may be metabolized during circulation before it reaches s.c. transplanted tumor. Possible metabolic pathways include conversion to the aldehyde by MAO, O-methylation by catechol O-methyltransferase, and nonspecific oxidation to the o-quinone form. To overcome this problem, we plan to administer 4-S-CAC more frequently in future *in vivo* experiments. Another approach is to protect either the o-dihydroxyl group by acetylation or phosphorylation (29) or the amino group by N-acetylation. The latter approach has been evaluated for 4-S-CAP in the form of N-acetyl-4-S-CAP, which showed some improvements in efficacy as an antimelanoma agent (30). Furthermore, combination therapy with buthionine sulfoximine was shown to enhance the antimelanoma activity of N-acetyl-4-S-CAP through the depletion of tissue glutathione (30). The same approach may also be applied to enhance the therapeutic efficacy of 4-S-CAC. In connection with the significance of tissue glutathione level, it should be noted that mouse melanoma

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**Table 3 Effects of cysteaminyldiphenols on the life span of mice who received i.p. B16 melanoma**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Median</th>
<th>Range</th>
<th>%ILS*</th>
<th>Body weight change (g)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>28.5</td>
<td>23–37</td>
<td>+1.1</td>
<td>+1.1</td>
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<tr>
<td>4-S-CAC-HCl</td>
<td>100</td>
<td>41.5</td>
<td>21–44</td>
<td>+1.3</td>
<td>+1.3</td>
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<tr>
<td>4-S-CAC-HCl</td>
<td>200</td>
<td>42.5</td>
<td>36–46</td>
<td>+0.6</td>
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<td>50</td>
<td>40.5</td>
<td>31–45</td>
<td>+2.2</td>
<td>+2.2</td>
</tr>
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<td></td>
<td></td>
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<td></td>
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<tr>
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<td>21.5</td>
<td>19–43</td>
<td>+1.1</td>
<td>+1.1</td>
</tr>
<tr>
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<td>20.5</td>
<td>19–45</td>
<td>+1.9</td>
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<td>22–29</td>
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<tr>
<td>DTIC</td>
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<td>28–37</td>
<td>+1.5</td>
<td>+1.5</td>
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<td>26–34</td>
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<tr>
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<td>21–31</td>
<td>+1.9</td>
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<td>40.0</td>
<td>37–45</td>
<td>+1.2</td>
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</table>

*a* On day 0, 5-week-old BDF₁ mice (10/group), 20–21 g, were given i.p. inoculations of 5 × 10⁶ B16 melanoma cells. Compounds were administered i.p. daily for 9 days starting on day 1.

* Percentage of increase in life span of treated versus control animals.

**Fig. 3. Effect of 4-S-CAC on growth rate of B16 melanoma in vivo.** Arrows at the bottom, times of injection. Treatment was initiated on day 8 after tumor inoculation. Control; ○, 300 mg/kg 4-S-CAP; ●, 100 mg/kg 4-S-CAC-HCl; □, 200 mg/kg 4-S-CAC-HCl; ▲, 50 mg/kg DTIC; 5 mice/group. Growth rates were expressed in log Vt/V₀, where Vt is the tumor volume at each measurement and V₀ is the tumor volume on day 8. *P < 0.05 from the control with the use of the Mann-Whitney nonparametric U test. 4-S-CAP (300 mg/kg) and 4-S-CAC-HCl (100 mg/kg) did not show a significant difference from the control on any measurement.

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cells become more sensitive to oxidative lysis through the glutathione
depletion (31).

In conclusion, the α-diphenol 4-S-CAC is a more potent antimelanoma
agent than its parent phenol 4-S-CAP. The tyrosinase (and TRP1)-
dependent cytotoxicity is suggested to be the major mechanism of anti-
melanoma action of 4-S-CAC. The oxidized α-quinone form of 4-S-CAC
may bind essential thiol enzymes such as thymidylate synthase (13, 17).
However, not all of the present results fit this hypothesis; we did not
observe a correlation between in vitro cytotoxicity and cellular melanin
content (Table 2). It is possible that, in the in vitro experiments, 4-S-CAC
might be oxidized by free iron present in the medium, and the resultant
α-quinone and/or active oxygen species could exert cytotoxicity.
Additional studies are certainly necessary to clarify the mechanism(s) of
cytotoxicity of this potentially promising antimelanoma agent and to
augment the cytotoxic effect of 4-S-CAC against melanoma.

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REFERENCES

2. Jiménez-Cervantes, C., Solano, F., Kobayashi, T., Urabe, K., Hearing, V. J., Lozano,
J. A., and García-Borrón, J. C. A new enzymatic function in the melanogenic
pathway: the 5,6-dihydroxyindole-2-carboxylic acid oxidase activity of tyrosine-
1969.
6. Wick, M. M., Byers, L., and Frei, E. α-Dopa: selective toxicity for melanoma cells in
7. Wick, M. M. 3,4-Dihydroxybenzylamine: a dopamine analog with enhanced antitu-
9. Prota, G., d’Ischia, M., and Mascagna, D. Melanogenesis as a targeting strategy
T. Selective toxicity of 5-S-cysteinyl-dopa, a melanin precursor, to tumor cells in vitro
11. Ito, S., Inoue, S., Yamamoto, Y., and Fujita, K. Synthesis and antitumor activity of
cysteinyln3,4-dihydroxyphenylalanines and related compounds. J. Med. Chem., 24:
12. Miura, S., Ueda, T., Jimbow, K., Ito, S., and Fujita, K. Synthesis of cysteinylnphenol,
cysteinylnphenol, and related compounds, and in vitro evaluation of antimelanoma
toxicity of 4-S-cysteinylnphenol and 4-S-cysteinylnphenol to melanocytes. Biochem.
14. Yamada, K., and Jimbow, K. Selective in vivo and in vitro incorporation and
accumulation of phenolic thioether amine into malignant melanoma and identification
15. Yamada, I., Seki, S., Ito, S., Suzuki, S., Matsubara, O., and Kasuga, T. The killing
effect of 4-S-cysteinylnphenol, a newly synthesized melanin precursor, on B16
effects of 4-S-cysteinylnphenol, a newly synthesized therapeutic agent specific to
17. Prezioso, J. A., Wang, N., and Bloomer, W. D. Thymidylate synthase as a target
enzyme for the melanoma-specific toxicity of 4-S-cysteinylnphenol and N-acetyl-
18. Miura, S., Jimbow, K., and Ito, S. The in vivo antimelanoma effect of 4-S-cyste-
20. Alena, F., Jimbow, K., and Ito, S. Melanocytotoxicity and antimelanoma effects
Antihypertensive activities of phenyl aminoethyl sulfides: a class of synthetic
22. Inoue, S., Ito, S., Wakamatsu, K., Jimbow, K., and Fujita, K. Mechanism of growth
inhibition of melanoma cells by 4-S-cysteinylnphenol and its analogues. Biochem.
J. A. Tyrosinase isoenzymes in mammalian melanocytes. I. Biochemical character-
ization of two melanosomal tyrosinases from B16 mouse melanoma. Eur. J.
25. Winder, A. J., and Harris, H. New assays for the tyrosine hydroxylase and dopa
26. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M., and
Ashton, B. J. Protocols for screening chemical agents and natural products against
in preventing metastases arising from intraocular melanomas in mice. Graefe’s Arch.
1990.
29. Pawelek, J. M., and Murray, M. Increase in melanin formation and promotion of
cytotoxicity in cultured melanoma cells by phosphorylated isomers of dopa. Cancer
30. Alena, F., Iwashina, T., Gil, A., and Jimbow, K. Selective in vivo accumulation of
N-acetyl-4-S-cysteinylnphenol in B16F10 murine melanoma and enhancement of its
in vitro and in vivo antimelanoma effect by combination of buthionine sulfoximine.
31. Peinado, P., Martínez-Liarte, J. H., del Marmol, V., Solano, F., and Lozano,
J. A. Glutathione depletion in mouse melanoma cells increases their sensitivity to oxidative
32. Ito, S., and Fujita, K. Microanalysis of eumelanin and phaeomelanin in hair and
melanomas by chemical degradation and liquid chromatography. Anal. Biochem.,
33. Ito, S., and Wakamatsu, K. An improved modification of permanganate oxidation
of eumelanin that gives a constant yield of pyrrole-2,3,5-tricarboxylic acid. Pigm.
Antimelanoma Effect of 4-S-Cysteaminylcatechol, an Activated Form of 4- S-Cysteaminylphenol

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