[CANCER RESEARCH 29, 1952–1955, November 1969]

# Inhibition of the Growth of Mouse Melanoma by Chlorpromazine[1]

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## SUMMARY

The effects of three phenothiazines, chlorpromazine (CPZ), chlorpromazine sulfoxide (CPZ-O), and 7-hydroxychlorpromazine (7-OH-CPZ), on the growth of B-16 and Harding-Passey mouse melanomas were investigated. CPZ and 7-OH-CPZ both inhibited tumor growth, but CPZ-O did not. The melanoma tyrosinase was activated by CPZ both *in vivo* and *in vitro*. There was no significant effect of CPZ on the succinic oxidase and reduced nicotinamide adenine dinucleotide oxidase of either the melanoma or liver of these animals. The marked affinity of phenothiazines for melanin might be used to develop more effective melanoma chemotherapeutic agents.

## INTRODUCTION

Chlorpromazine (CPZ) has been demonstrated to have a marked affinity for melanin-containing tissues in animals and man. In 1962, Potts (14) demonstrated that the uveal tracts of pigmented rabbits concentrated $^{35}$S-labeled CPZ in amounts 50 times greater than the other body tissues; this did not occur in albino animals. The preferential localization of CPZ in the melanized iris and choroid was also demonstrated in man at autopsy (9). When investigating the accumulation of $^{35}$S-labeled CPZ in melanoma-bearing mice, Blois (4) found the radioactivity most persistent in eye and tumor tissues.

Phenothiazine compounds also interfere with various enzymatic reactions. CPZ *in vitro* has been demonstrated to uncouple oxidative phosphorylation and to inhibit the oxidation of several tricarboxylic acid substrates in the liver and brain (1, 3, 7). The localization of CPZ in melanin-bearing tissues and its enzyme-inhibitory properties suggested the possibility that chronic administration of this drug might interfere selectively with the metabolism of malignant melanoma.

In the present study, CPZ and two of its metabolites, 7-hydroxychlorpromazine (7-OH-CPZ) and chlorpromazine sulfoxide (CPZ-O), were administered to C57BL/6J mice bearing B-16 melanomas. Measurements of tumor volumes and tumor weights indicated that CPZ and 7-OH-CPZ had significant growth-inhibitory effect. The effect of CPZ on tyrosinase, succinic oxidase, and reduced nicotinamide adenine dinucleotide (NADH) oxidase activities in the B-16 mouse melanoma was also measured.

## MATERIALS AND METHODS

B-16 and Harding-Passey mouse melanomas were obtained from the Jackson Laboratories, Bar Harbor, Maine. The tumors were serially transplanted subcutaneously into C57BL/6J and BALB mice respectively.

Chlorpromazine and chlorpromazine sulfoxide were obtained from Smith Kline & French Laboratories, Philadelphia, Pa., and 7-hydroxychlorpromazine was obtained from Dr. Albert A. Manian, Psychopharmacology Research Branch, National Institute of Mental Health, Bethesda, Maryland.

**Experimental Procedure.** The drugs were dissolved in 0.2 ml of physiologic saline and administered by intraperitoneal injection once daily, five days a week, beginning two days after transplantation of the tumor in the B-16 melanoma and seven days after transplantation in the Harding-Passey melanoma. The first three daily injections of CPZ consisted of 10 mg/kg to develop tolerance to the sedation produced by the higher doses of CPZ. Control mice were injected with 0.2 ml of physiologic saline on an identical schedule. Serial total-body weights were recorded and tumor size was measured either by weight of the dissected tumor or by volume calculated using external caliper measurements according to the formula $V = \pi/4(d_1)(d_2)(d_3)$. Sections of melanoma tissues were placed in formalin for light microscopic examination. The weights of all mice were recorded at the beginning, during, and at the end of the experiment.

**Enzyme Assays.** On the 11–13th day after transplantation of the B-16 melanoma, 6–8 mice from each group were killed by decapitation. Older tumors were avoided because they usually developed central necrosis. The melanomas and livers were excised and homogenized in a Potter-Elvehjem homogenizer with 10 volumes of 0.25 M sucrose at 4°C. Enzyme activities were measured with a Biological Oxygen Monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) which employs an oxygen electrode. Oxygen consumption was calculated from the change in oxygen concentration in a solution initially saturated with air at 37°C. Succinic oxidase and NADH oxidase were determined by an adaptation of the procedure given by Umbreit *et al.* (16). The effect of CPZ on the enzyme tyrosinase was evaluated by assaying both the monophenol (tyrosine-tyrosinase) and diphenol (dopa-tyrosinase) oxida-

[1] This work was supported by USPHS Grant NB07542-01.
Received January 21, 1969; accepted March 27, 1969.

tion. Each reaction vessel contained 0.1 M potassium phosphate buffer, pH 6.8, 0.5 ml 10% melanoma homogenate, and either 1.0 mg of a 10:1 mixture of L-tyrosine and L-dopa (tyrosine-tyrosinase) or 1.0 mg of L-dopa (dopa-tyrosinase) as substrate. The total volume of each reaction mixture was 3 ml, and the temperature was maintained at 37°C. Protein content of the homogenates was measured by the method of Lowry *et al.* (12).

## RESULTS

Chart 1 shows the effects of CPZ and CPZ-O on the growth of B-16 melanoma. The injections were initiated on the second day after transplantation of the tumor. CPZ, 19 mg/kg/day (5 days a week), produced an inhibition of tumor growth as measured by tumor volume. The first appearance of the tumor after transplantation was delayed several days in the CPZ-treated group compared to the CPZ-O-treated and control animals. CPZ-O (25 mg/kg) had no effect on the size of the melanoma. Since after several weeks many of the tumors in both groups became ulcerated or were nibbled on and regressed, it was impossible to evaluate the effect of CPZ on the survival time. Chart 2 shows that there was total-body weight loss after the 20th day of the experiment in the CPZ-treated animals, whereas the control and CPZ-O groups gained weight. The average weights of the excised melanomas in the control and CPZ-treated mice sacrificed on the 18th day after transplantation are given in Table 1. CPZ (19 mg/kg/day) reduced the average weight of the tumors to approximately one third of the control values in the B-16 melanoma. The average weight of the tumors in the group receiving 7-OH-CPZ was about 50% less than that in the saline-injected control group (Table 1).

Since CPZ produced a sedative effect in these animals, sodium pentobarbital was administered under identical conditions to evaluate the effect of sedation on the growth of B-16 melanoma. This barbiturate had no significant effect on the tumor growth (Table 1). Both pentobarbital and CPZ caused a decrease in total-body weight of the animals.

The results of treatment with CPZ on the tumor weight of the Harding-Passey melanoma are also seen in Table 1. Since this melanoma is a slower-growing tumor, the CPZ injections were not initiated until one week after transplantation of the tumor. The weights of the treated tumors in this group were reduced to 38% of the untreated group; this finding was similar to that seen with the B-16 melanoma.

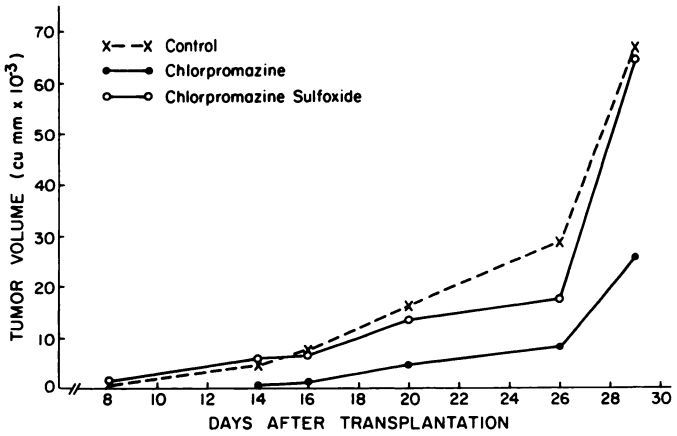


Chart 1. Effect of chlorpromazine and chlorpromazine sulfoxide on the tumor volumes of B-16 melanoma. Each point on the graph is the mean of 35 tumors in the chlorpromazine-treated and control groups. Chlorpromazine sulfoxide values are the mean of 10 tumors. Intraperitoneal injections of the drug (5 days per week) were initiated 2 days after transplantation of the tumor.

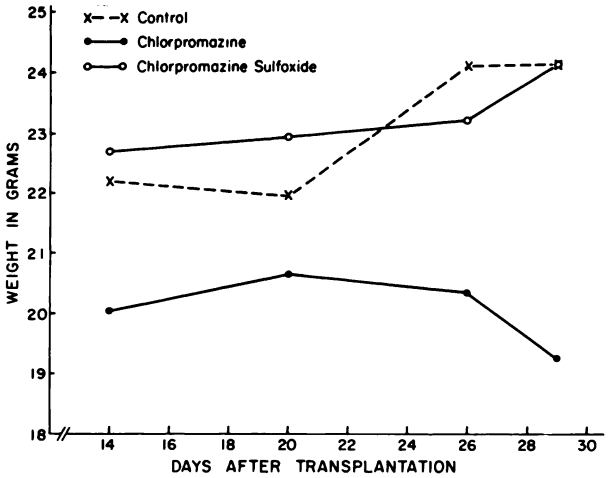


Chart 2. Effect of chlorpromazine and chlorpromazine sulfoxide on the body weights of the mice used in Chart 1.

Table 1

| Drug | Dose (mg/kg/day) | Body wt. change (gm) | Wt. of tumor (mg) | T/C[a] |
|---|---|---|---|---|
| B-16 Melanoma | | | | |
| Controls (20) | | +2.7 | 3063 | |
| CPZ (20) | 19 | −1.9 | 1015 | 0.33 |
| Controls (30) | | +0.8 | 2590 | |
| 7-OH-CPZ (5) | 15 | | 1320 | 0.51 |
| CPZ (5) | 15 | | 958 | 0.37 |
| CPZ (5) | 19 | −2.0 | 754 | 0.29 |
| Sodium pentabarbital (10) | 45 | −1.0 | 2356 | 0.91 |
| Harding-Passey Melanoma | | | | |
| Controls (15) | | −0.5 | 1661 | |
| CPZ (15) | 12.5 | −2.7 | 637 | 0.38 |

Effect of drugs on mouse melanomas. The numbers in parentheses indicate the number of animals. Drugs were administered five times a week. In the B-16 melanoma, injections were started 2 days after transplantation of tumors, and the tumors were removed and weighed 18 days after transplantation. In the Harding-Passey melanomas, injections were started 7 days after transplantation of tumors, and the tumors were removed and weighed 26 days after transplantation. CPZ, chlorpromazine; 7-OH-CPZ, 7-hydroxychlorpromazine.

[a] $T/C = \frac{\text{weight of treated tumors}}{\text{weight of control tumors}}$.

Table 2 shows the *in vivo* effect of CPZ on various enzymes in the B-16 melanoma. When compared with their saline-injected controls, the melanomas of mice treated with CPZ displayed increased activities of tyrosine-tyrosinase and dopa-tyrosinase. Succinic oxidase and NADH oxidase were essentially unchanged in both the melanoma and liver of these two groups of animals.

The increased activity of tyrosinase in the CPZ-treated animals could be due to either induction or activation of the enzyme. We therefore investigated the effect of CPZ on the *in vitro* oxidation of tyrosine and dopa. As seen in Table 3, $10^{-3}$ M CPZ *in vitro* produced a similar percentage increase in tyrosinase to that seen *in vivo*. Although this concentration of CPZ is higher than that reported in the brain and liver tissues, the preferential accumulation of CPZ in melanoma may explain these findings. Both the oxidation of the monophenolic substrate (tyrosine) and dehydrogenation of the diphenolic substrate (dopa) were activated to a similar degree by CPZ *in vivo* and *in vitro*. CPZ had no effect on the oxygen uptake of the blanks containing either homogenate without substrate or substrate alone.

Light microscopic examination of H & E stained sections of melanomas from both control and CPZ-treated animals revealed no pathologic changes due to the drug.

## DISCUSSION

CPZ and 7-OH-CPZ were found to inhibit the growth of mouse B-16 melanoma, while CPZ-O did not. The growth of the Harding-Passey melanoma was also inhibited to a similar degree by CPZ. CPZ has been demonstrated to have an antineoplastic activity in some animal tumors and none in others. In 1957, Belkin and Hardy (2) reported that CPZ had an inhibiting effect on the growth of mouse Sarcoma 37 tumors. However, toxic levels of the drug were necessary to produce significant growth inhibition. Subsequent studies indicated that CPZ has a carcinostatic effect on mastocytoma (11) but not on Ehrlich ascites carcinoma (5) or mouse mammary adenocarcinoma (6). The mechanism of action of CPZ on these susceptible tumors has not been ascertained. The sensitive tumors may concentrate toxic levels of CPZ in their cells, or perhaps the drug might have a specific metabolic

Table 2

| Enzymes | Melanoma $\mu l\ O_2$/hr/gm protein | | Liver $\mu l\ O_2$/hr/gm protein | |
|---|---|---|---|---|
| | Control | CPZ-treated | Control | CPZ-treated |
| Succinic oxidase (5) | 15,328 ± 2,060 | 18,205 ± 2,451 | 63,787 ± 6,390 | 57,944 ± 7,148 |
| NADH oxidase (5) | 2,270 ± 564 | 2,187 ± 396 | 12,087 ± 2,031 | 9,557 ± 1,323 |
| Tyrosine-tyrosinase (12) | 1,189 ± 131 | 1,945 ± 177[a] | | |
| Dopa-tyrosinase (8) | 2,215 ± 286 | 4,404 ± 381[b] | | |

Enzyme activities in the B-16 melanoma and liver homogenates of chlorpromazine (CPZ)-treated and control mice. The CPZ-treated animals received 19 mg/kg i.p. 5 days per week starting the second day after transplantation of the tumors. The control group received saline on an identical schedule. The melanoma and liver were removed on the 11th to 13th day after transplantation. Reaction mixture for succinic oxidase: sodium phosphate buffer, 0.033 M, pH 7.4; sodium succinate, 0.05 M; cytochrome *c* $1.3 \times 10^{-5}$ M; $CaCl_2$, $4 \times 10^{-4}$ M; $AlCl_3$, $4 \times 10^{-4}$ M; and 0.1 ml of a 10% tissue homogenate. Reduced nicctinamide adenine dinucleotide ($2 \times 10^{-3}$ M) replaced the sodium succinate in the reduced nicotinamide adenine dinucleotide oxidase assay. The tyrosinase assay is described in the *Materials and Methods*. Final reaction volume, 3.0 ml; gas phase, air; temperature, 37°C. Each assay was run in duplicate, and the numbers in parentheses indicate the number of animals. The values are expressed as the mean ± S. E.

[a] $P < 0.005$.
[b] $P < 0.001$.

Table 3

| | Tyrosine-tyrosinase | | Dopa-tyrosinase | |
|---|---|---|---|---|
| | $\mu l\ O_2$/hr/gm of protein | % of control | $\mu l\ O_2$/hr/gm of protein | % of control |
| Control (4) | 1002 ± 50 | | 2666 ± 271 | |
| CPZ (4) $5 \times 10^{-4}$ M | 1576 ± 42[a] | 157 | 3740 ± 371[b] | 140 |
| CPZ (4) $10^{-3}$ M | 1800 ± 98[a] | 180 | 5213 ± 481[c] | 196 |
| CPZ (4) $1.5 \times 10^{-3}$ M | 2327 ± 106[a] | 232 | 6021 ± 565[a] | 226 |

*In vitro* effect of chlorpromazine (CPZ) on tyrosinase activity of B-16 melanoma. The reaction mixtures and conditions were identical to those in Table 2.

[a] $P < 0.001$.
[b] $P < 0.1$.
[c] $P < 0.005$.

action in these tumors. It has been established that CPZ accumulates in higher concentrations in melanoma than in nonmelanized tissues (4). This preferential concentration of CPZ in melanoma may have toxic effects on the metabolism of the melanocyte. High concentrations of CPZ uncouple oxidative phosphorylation and inhibit hepatic and brain succinic oxidase and NADH oxidase *in vitro* (1, 3, 7). CPZ also inhibits the catalytic oxidation of NADH by melanin (17), which could disturb the metabolism of the melanocyte. However, our data indicate that succinic oxidase and NADH oxidase were similar in the CPZ-treated and control melanomas. Another biologic effect of CPZ is the inhibition of DNA synthesis in bone marrow granulocytes, which results in prolonged generation time and delayed cell division (13). Whether DNA synthesis in melanoma is also inhibited by CPZ has not been ascertained.

Demopoulas (8) has proposed that tyrosinase activity might be necessary to the energy metabolism of the melanoma cell. He attributes the growth-inhibitory effect of phenyl lactate and penicillamine on S91 mouse melanoma to the ability of these compounds to inhibit tyrosinase (8). On the other hand, our results demonstrate that the carcinostatic action of CPZ is associated with an increase in tyrosinase activity in the B-16 melanoma.

This increase in enzyme activity was also seen when CPZ was added *in vitro*. Most of the tyrosinase activity in the B-16 melanoma is present in the melanosomes, and melanin synthesis occurs within these organelles (15). CPZ combines with the melanosomes by forming a charge-transfer complex with the melanin (10). The activation of tyrosinase may be related to the close proximity of CPZ to the enzyme. The cutaneous hyperpigmentation seen in patients treated with large doses of CPZ may be secondary to tyrosinase activation.

The potential clinical value of any cancer chemotherapeutic agent depends on its toxic side effects and selective action on the tumor tissue. The per kilogram dose of CPZ used in these animals is comparable to that commonly used to treat severe mental disturbances in man. As much as 2000 to 5000 mg of CPZ a day have been administered to patients without causing serious side effects. Our results suggest that toxic levels of CPZ selectively accumulate in mouse melanoma. Continued efforts to find or develop melanin-seeking phenothiazines which also have carcinolytic action might be a useful approach to melanoma therapy.

## ACKNOWLEDGMENTS

The authors wish to thank Smith Kline & French Laboratories and Dr. Albert Manian for the generous donations of compounds used in these studies. We gratefully acknowledge the technical assistance of Miss Frances Korb.

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*Cancer Res* 1969;29:1952-1955.