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Ambiguous Effect of Chlorpromazine on Doxorubicin Activity Against P388D$_1$ Tumours in Mice

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Abstract—*Chlorpromazine (CPZ) can decrease the toxicity of doxurubicin (DOX). We wanted to determine whether this CPZ pretreatment could affect the response of tumours to therapeutic doses of DOX. Six groups of eight female CDF$_1$ mice received 1 million leukaemia P388D$_1$ cells i.p. For 5 days, they received DOX i.p. (total dose 0, 6 or 12 mg/kg), preceded by saline or 5 mg/kg CPZ s.c. CPZ in the absence of DOX had no effect on survival [median survival time (MST) of 19 days vs. 20]. In mice receiving DOX only, MST was > 60 days. Mice receiving DOX + CPZ were similar to DOX till day 25, but subsequently died earlier (MST of 27 and 34 days, for DOX 6 and 12 mg/kg). At death or day 60, 31% (5/16) of DOX mice and 88% (14/16) of DOX + CPZ had macroscopic tumours (P < 0.005, both DOX dose groups combined). However, only 19% (3/16) of DOX and 6% (1/16) of DOX + CPZ had tumours in the abdominal cavity, the others being in the abdominal wall close to the site of injection. The results suggests that while CPZ did not affect the killing of cancer cells in the abdominal cavity, it did block the effect of DOX on cells in the abdominal wall and skin. This block may be caused by decreased local delivery of DOX, due to the hypothermia produced by CPZ.*

INTRODUCTION

DOXORUBICIN is a highly effective drug for treating cancer [1, 2]; however, its usefulness is limited by its toxicity [3, 4].

We had previously shown that doxorubicin toxicity can be decreased if mice are pretreated with chlorpromazine [5]. However, our results did not tell us whether in these mice, doxorubicin would still be effective against cancer cells.

To answer this question we used a cancer model known from previous experience to be responsive to anthracyclines: CDF$_1$ mice injected with P388D$_1$ leukaemia cells (6).

We found that chlorpromazine pretreatment did not abolish the therapeutic effect of doxorubicin against the principal tumour, but did promote the survival of usually minor tumours in the skin.

METHODS

CDF$_1$ female mice (Charles River, Montreal) weighing 17–20 g were acclimatized to the animal house for 1 week. Food and water were freely available throughout. They were injected i.p. with 1,000,000 P388D$_1$ leukaemia cells in 0.5 ml physiological saline. The next day, the mice were randomly assigned to six groups of eight each; mean weights for the groups were between 17.8 and 18.2 g. Each group received chlorpromazine and doxorubicin–HCl for the next 5 days according to the following schedule:

Group	CPZ	DOX (mg/kg)
1	−	0
2	+	0
3	−	6
4	+	6
5	−	12
6	+	12

Each day of the treatment, chlorpromazine (5 mg/kg) was given s.c. in 0.2 ml saline per 18 g. One hour later, the mice received one-fifth of the above doxorubicin dose i.p. (i.e. 1.2 or 2.4 mg/kg) in 1.5 ml saline per mouse. All solutions were

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Table 1. *Median survival time (days)*

Doxorubicin (mg/kg)	Chlorpromazine −	Chlorpromazine +
0	20	19
6	>60	27
12	>60	34

prepared immediately prior to use. Doxorubicin–HCl (Adriamycin) was graciously provided by Adria Laboratories of Canada Ltd., Mississauga, Ontario. Chlorpromazine–HCl (Largactil) was obtained from Rhone-Poulenc Pharma Inc., Montreal, Quebec. Doses are expressed as the free base for chlorpromazine, and as the HCl salt for doxorubicin. The source of the leukaemia cell line was American Type Culture Collection No. CCL46.

The mice were kept at 23°C throughout. Preliminary experiments and previous experience [5, 7] indicate that the rectal temperature of the mice reaches about 32°C at the time of doxorubicin injection. It reaches a minimum of 28°C within 4 h, and recovers completely by 10 h. The effect of chlorpromazine decreased slightly with repeated injections, but the temperature still dropped to a 31–32°C minimum on the last day of the treatment. Doxorubicin had no acute effect on temperature.

Surviving mice were sacrificed after 60 days. All mice were examined for the presence of macroscopic tumours (at least 3 mm in diameter). The locations of any that were found were recorded.

RESULTS

1. *Control animals*

All the animals that did not receive doxorubicin developed large tumours in the abdominal cavity, and all had died by day 23, with a median survival time of 20 days (Fig. 1, Table 1). Chlorpromazine had no effect on either the survival (Fig. 1) or weight gain (Fig. 2) of these animals.

2. *Doxorubicin-treated animals*

There were no statistically significant differences in any of the parameters between the 6 and 12 mg/kg dose groups; consequently, they are considered together in most of the subsequent discussion.

The survival of animals treated with either dose of doxorubicin was greatly extended. None died prior to day 19, and median survival time was greater than 60 days. After 60 days, 62% of the mice (6/8 of the 6 mg/kg DOX treated and 4/8 of the 12 mg/kg treated) were alive (compared to no doxorubicin: $P < 0.025$, chi-square test).

3. *Doxorubicin and chlorpromazine treatment*

The mortality of doxorubicin-treated mice receiving the chlorpromazine pretreatment was very similar to that of the mice receiving only doxorubicin till day 27, but increased subsequently (Fig. 1, Table 1). After 60 days, only one mouse (Group 6) was alive ($P < 0.05$, chi square).

4. *Location of tumours*

When the doxorubicin-treated animals were examined, an effect of chlorpromazine was seen both on the number and the location of the tumours (Table 2). Sixty-nine per cent (11/16) of the doxorubicin treated animals were tumour free; however, of those that also received chlorpromazine, only one (6%) was tumour free ($P < 0.01$, chi-square).

However, there was no significant difference in the number of mice with tumours inside the abdominal cavity (3/16 for doxorubicin only and 1/16 for chlorpromazine + doxorubicin); the difference in total tumours was accounted for by the greater number of tumours in the abdominal wall in the mice receiving chlorpromazine (13/16 vs. 2/16, $P < 0.001$).

5. *Body weight*

After 1 week, the weight of the untreated mice increased very rapidly as their tumours grew (Fig. 2). Immediately following the treatment (days 7 and 10), the mice which had received chlorpromazine plus doxorubicin (both doses) were significantly lighter than the mice receiving doxorubicin only ($P < 0.02$, 2-tailed Student's t-test), but the difference was not maintained. No such difference was seen between groups 1 and 2 (no doxorubicin).

DISCUSSION

Although this dose of chlorpromazine had no effect on tumour growth by itself, it clearly increased the incidence of tumours in mice treated with doxorubicin.

The tumours formed solid masses and were found in only two locations in the mice: inside the abdominal cavity, where the cancer cells were injected, and in the lower abdominal wall and skin close to the site of injection. Since there was no trace of tumours in sites remote from the injection site it seems likely that the tumours in the skin were derived from cells which had been implanted there during injection; none were found in sites untouched by the needle.

The incidence of tumours in the abdominal wall and skin of the doxorubicin treated mice was greatly increased by chlorpromazine. It is likely that these tumours were also present in the mice not receiving doxorubicin, but did not become prominent before the mice died of their intraperitoneal tumours. However, our results do not enable us to totally eliminate the possibility of tumour promotion by

Table 2. Site of tumours

Tumour site	Number of mice					
	DOX 0		DOX 6		DOX 12	
	−CPZ	+CPZ	−CPZ	+CPZ	−CPZ	+CPZ
Tumours present						
Abdominal cavity	8	8	1	1	2	0
Abdominal wall only	—	—	1	6	1	7
Tumour-free at 60 days	—	—	6	1	5	1
Mouse dead	—	—	1	1	1	0
Mouse alive	—	—	5	0	4	1

DOX 0, DOX 6, DOX 12: total doxorubicin dose in mg/kg.

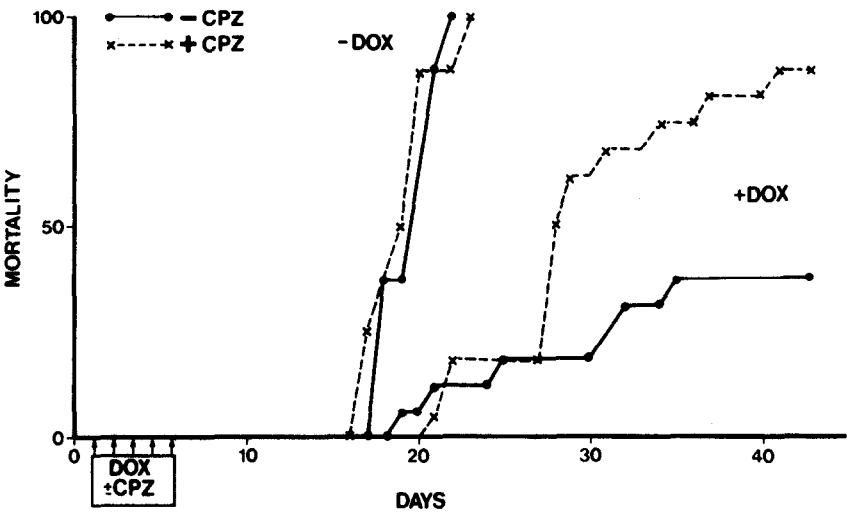

Fig. 1. Effect of doxorubicin (DOX) and chlorpromazine (CPZ) on % mortality of tumour-bearing mice. The two DOX dose groups were combined for this graph.

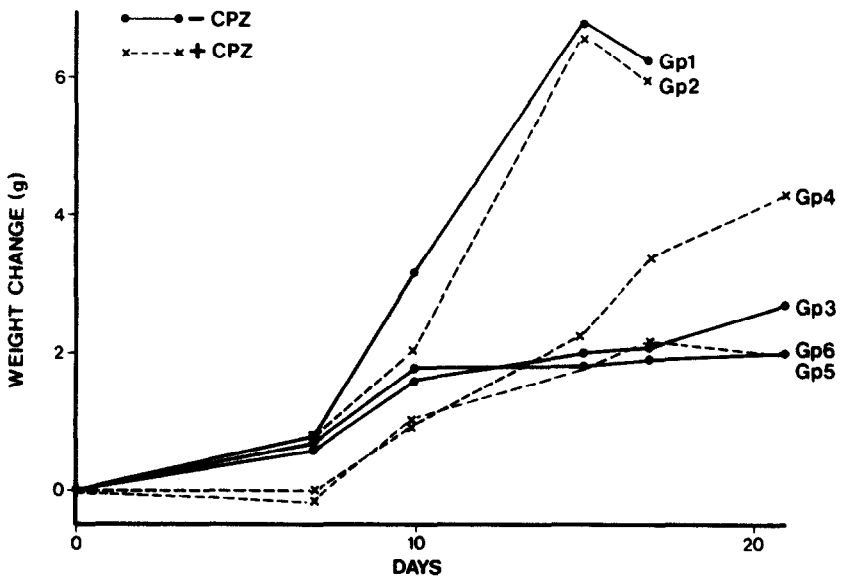

Fig. 2. Effect of doxorubicin and chlorpromazine treatment on body weight. Doxorubicin doses were 0 mg/kg *for groups* 1 *and* 2, 6 mg/kg *for groups* 3 *and* 4, *and* 12 mg/kg *or groups* 5 *and* 6. *Chlorpromazine-treated groups are shown by dashed lines. The weight increase in group* 4 *was due to a large tumour in one mouse and was not statistically significant. The curves were discontinued when more than* 25% *of a group were dead.*

chlorpromazine.

The protection that these cells enjoy against doxorubicin is probably due to the hypothermia that chlorpromazine produces rather than a direct cellular effect. We base this conclusion on the numerous reports of chlorpromazine increasing rather than decreasing doxorubicin efficacy in isolated cells [8, 9]. Resistant cells in particular can be rendered more vulnerable to doxorubicin by chlorpromazine and other phenothiazines [10]. It has also been suggested that chlorpromazine may have a doxorubicin-like effect [11].

Hypothermia, on the other hand, decreases the efficacy of doxorubicin. Chlorpromazine hypothermia protects mice against doxorubicin toxicity [5], and hypothermia has been suggested as a mechanism in protection by adenosine [12]. Chromosome abberations due to doxorubicin are also decreased by hypothermia [13]. Conversely, hyperthermia increases doxorubicin toxicity to isolated cells [14, 15], possibly by increasing its uptake]16].

In the whole animal, hyperthermia can increase doxorubicin toxicity and decrease the subsequent survival of cancer cells [17].

The lack of an effect on skin tumours is probably due to the skin being cooler than the core. This would be accompanied by decreased flow, as well as decreased doxorubicin uptake. The results may be directly related to the protection against hair loss during doxorubicin treatment obtained by cooling of the scalp [18]. The caveat that scalp cooling should not be used when there is a possibility of local metastases [19] may also apply to chlorpromazine.

Chlorpromazine did not prevent the killing of intraperitoneal cancer cells, since the incidence of tumours in this location was not increased. However, since similar effects were obtained by treatment with 6 and 12 mg/kg doxorubicin, it is possible that the lower dose of doxorubicin was already maximally effective. If this were the case, even a sizeable decrease in the efficacy of doxorubicin may not become evident. The experiments would have to be extended to a lower dose to resolve the question.

Similarly, an effect of chlorpromazine on doxorubicin toxicity could not be demonstrated; doxorubicin toxicity was low in our experiment, at least partially because of the large injection volume used (one mouse each of groups 3, 4, and 5 died without visible tumours on days 19–25).

The decreased body weight of the mice following chlorpromazine treatment could be explained by decreased food intake during tranquilization, although this would not explain why no difference is seen in mice not receiving doxorubin; it could be due to doxorubicin-induced anorexia or to tumour growth.

In conclusion, within the design of the experiment chlorpromazine treatment decreased the efficacy of doxorubicin against disseminated tumours, but did not affect the suppression of intraperitoneal tumours.

The results suggest that in the case of disseminated tumours, the use of chlorpromazine with doxorubicin should be carefully weighed, particularly if hypothermia is present.

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REFERENCES

1. Blum RH. Overview of studies with 'adriamycin' (NSC-123127) in United States. *Cancer Chemother Rep* 1975, **6**, 247–251.
2. Goodman TL, Lochner J. Antibiotics in cancer chemotherapy: the anthracyclines. *Hosp Formul* 1986, **21**, 444–455.
3. Minow RA, Benjamin RS, Gottlieb JA. Adriamycin (NSC-123127) cardiomyopathy. Overview with determination of risk-factors. *Cancer Chemother Rep* 1975, **6**, 195–201.
4. Henderson IC, Frei III E. Adriamycin cardiotoxicity. *Am Heart J* 1980, **99**, 671–674.
5. Gailis L. Protection against doxorubicin toxicity in mice by chlorpromazine hypothermia. *Res Commun Chem Pathol Pharmacol* 1986, **53**, 319–330.
6. Lagueux J, Page M, Delorme F. Daunorubicin–albumin copolymer targeting to leukemic cells *in vitro* and *in vivo*. *Semin Oncol* 1984, **11** (Suppl. 3), 59–63.
7. Gailis L, Tourigny DEC. Chlorpromazine and dithioerythritol protection against acute ethanol toxicity. *Alcoholism Clin Exp Res* 1984, **8**, 308–313.
8. Krishan A, Bourguignon LYW. Cell cycle related phenothiazine effects on adriamycin transport. *Cell Biol Int Rep* 1984, **8**, 449–456.
9. Dalmark M, Hoffmann EK. Doxorubicin (Adriamycin) transport in Ehrlich ascites tumour cells: comparison with transport in human red blood cells. *Scand J Clin Invest* 1983, **43**, 241–248.
10. Ganapathi R, Grabowski D, Turinic R, Valenzuela R. Correlation between potency of calmodulin inhibitors and effects on cellular levels and cytotoxic activity of doxorubicin (Adriamycin) in resistant P388 mouse leukemia cells. *Eur J Cancer Clin Oncol* 1984, **20**, 799–806.
11. Darkin S, McQuillan J, Ralph RK. Chlorpromazine: a potential anticancer agent? *Biochem Biophys Res Commun* 1984, **125**, 184–191.

12. Newman RA, Hacker MP, Krakoff IH. Amelioration of adriamycin and daunorubicin myocardial toxicity by adenosine. *Cancer Res* 1981, **41**, 3483–3488.
13. Vig BG. Effect of hypothermia and hyperthermia on the induction of chromosome aberrations by adriamycin in human leukocytes. *Cancer Res* 1978, **38**, 550–555.
14. Block JB, Harris PA, Peale A. Preliminary observations on temperature-enhanced drug uptake by leukemic leukocytes *in vitro*. *Cancer Chemother Rep* 1975, **59**, 985–988.
15. Hahn GM, Strande DP. Cytotoxic effects of hyperthermia and adriamycin in Chinese hamster cells. *J Natl Cancer Inst* 1976, **57**, 1063–1069.
16. Hahn GM, Braun J, Har-Kedar I. Thermochemotherapy: synergism between hyperthermia (42–43°) and adriamycin (or bleomycin) in mammalian cell inactivation. *Proc Natl Acad Sci USA* 1975, **72**, 937–940.
17. Marmor JB, Kozak D, Hahn GM. Effects of systemically administered bleomycin or adriamycin with local hyperthermia on primary tumor and lung metastases. *Cancer Treat Rep* 1979, **63**, 1279–1290.
18. Luce KL, Raffetto TJ, Crisp IM, Grief GC. Prevention of alopecia by scalp cooling of patients receiving Adriamycin. *Cancer Chemother Rep* 1973, **57**, 108–109.
19. Dean JC, Salmon SE, Griffith KS. Prevention of doxorubicin-induced hair loss with scalp hypothermia. *N Engl J Med* 1979, **301**, 1427–1429.