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Inhibition of thermotolerance by phenothiazines in *Escherichia coli* B/r

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Chlorpromazine and trifluoperazine, which are known potentiators of the hyperthermic effect both *in vitro* and *in vivo* inhibited development of thermotolerance in *E. coli* B/r. In addition, the presence of these drugs during hyperthermia resulted in thermosensitization of normal as well as thermotolerant cells.

THE use of hyperthermia in cancer therapy either alone or in combination with other treatments is currently gaining importance. However, development of thermotolerance in tissues during fractionated hyperthermic treatments is a serious drawback. This temporary resistance towards heat is induced by heat shock and is an adaptive process triggered during initial thermal stress and developing over a period of time^{1,2} when the cells are held at 37°C, indicating the necessity of cellular metabolism for its expression³. In fact, the synthesis of heat shock proteins (HSP) has been generally correlated with the appearance of thermotolerance, though there are several exceptions⁴. Hence development of thermotolerance could be overcome by inhibiting protein synthesis in general⁵ and HSP synthesis in particular.

Phenothiazine derivatives chlorpromazine (CPZ) and trifluoperazine (TFP) have been demonstrated to potentiate the effects of hyperthermia both *in vitro*⁶ and *in vivo*⁷. Since they are also inhibitors of protein synthesis, their

role in the development of thermotolerance was investigated.

E. coli B/r cells were grown overnight at 37°C in nutrient broth (Difco). The washed cells, resuspended in phosphate-buffered saline (PBS, pH 7.0) at concentration of 10⁸ ml⁻¹, were used in all the studies. Heat treatment was given in a water-bath shaker with a temperature accuracy of 0.1°C. CPZ and TFP were used as supplied by Sigma, St Louis, USA.

Preliminary investigations were carried out to observe the effect of initial thermal shock at temperatures varying from 40 to 45°C. The resultant cellular lethality in control cells as well as in the presence of either CPZ or TFP at concentrations varying from 2 to 20 µM for a fixed period of 60 min was ascertained. A temperature which was nonlethal to the cells in the presence and absence of a particular concentration of CPZ or TFP was thus selected.

Incubation at 37°C following the initial thermal shock was varied from 30 to 180 min to determine the development of maximum thermotolerance. The temperature for subsequent heat treatment (evaluation of tolerance) was the one at which there was about 10% survival in control cells after a 90-min exposure.

Based on these preliminary investigations, the following protocol was adhered to in all the subsequent investigations: Initial heat shock at 42°C for 60 min with or without either CPZ (10 µM) or TFP (5 µM) + incubation at 37°C for 120 min + re-exposure at 50°C for up to 90 min. At the end of the protocol, the cells were suitably diluted in PBS, plated on nutrient agar and incubated at 37°C for 18 h, and the colonies counted. All the experiments were carried out at least thrice with replicate plates at two plating dilutions. Mean percentage survival was plotted against duration of re-exposure.

Heating the cells at 42°C was nonlethal to both control cells and cells treated with CPZ (10 µM) or TFP (5 µM). Control cells heat-shocked for 60 min at 42°C and subsequently incubated for 120 min at 37°C demonstrated maximum thermotolerance to the challenge at 50°C. As shown in Table 1, the time required for 50% lethality at 50°C (TD₅₀) was 32 min, as against 68 min for cells with fully developed thermotolerance. There was no further development of thermotolerance in cells when the time of incubation was extended to 180 min (data not

Table 1. Effect of phenothiazine derivatives on thermal response in *E. coli* B/r.

Treatment	Time required (min) for 50% lethality when exposed to 50°C		
	Control	CPZ	TFP
Control (heat shock, no incubation, no drug)	32	—	—
Control, with drug	—	15	16
Heat shock, 37°C incubation, no drug	68	—	—
Heat shock, 37°C incubation, with drug	—	20	22
Drug washed off before incubation	—	68	68
Drug washed off after incubation	—	31	33
Drug added before incubation	—	31	33
Drug added after incubation	—	31	33

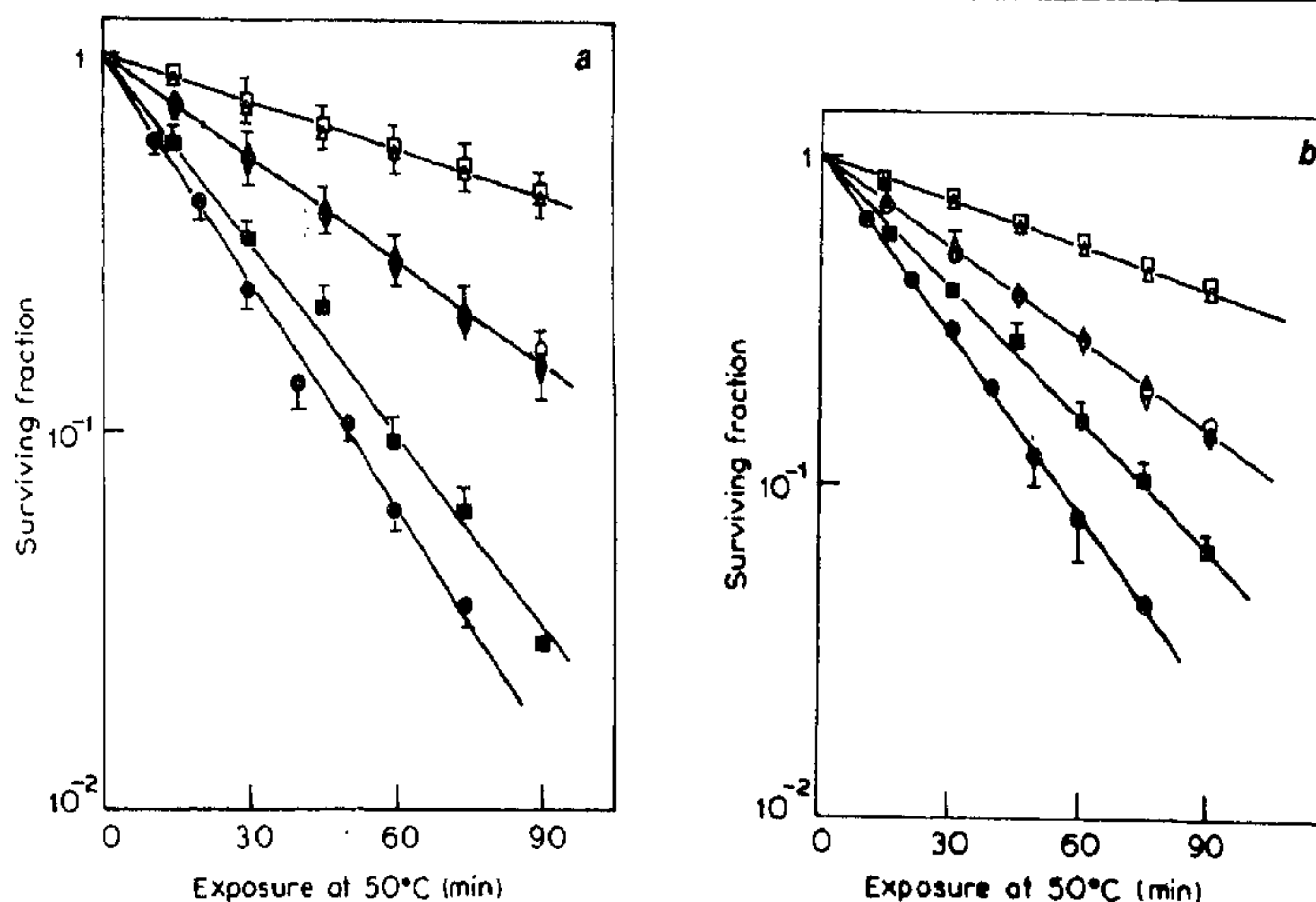


Figure 1. Effect of (a) chlorpromazine and (b) trifluoperazine on thermal response (50°C) in *E. coli* B/r. ○, Control, no drug; ●, Control, with drug; □, heat-shocked and 37°C incubated cells, no drug; ■, heat-shocked and 37°C incubated cells, with drug; △, drug washed off before incubation; ▲, drug washed off after incubation; ▽, drug added before incubation; ▼, drug added after incubation.

shown).

CPZ, when added to cells immediately prior to heat shock and present continuously during subsequent incubation and challenge, reduced cell survival to 50% (TD_{50}) at the end of 20 min exposure at 50°C (Table 1 and Figure 1a). A similar effect was noticed in the presence of TFP; TD_{50} was 22 min (Figure 1b). No pretreatment of the cells with the drugs was necessary (data not shown). TD_{50} for cells exposed to 50°C with drug but without prior heat shock and incubation was 15 min with CPZ and 16 min with TFP, indicating thermosensitization. When the drug was washed off after the heat shock (i.e. before incubation), the cells became thermotolerant (TD_{50} = 68 min), but adding the drug before incubation resulted in inhibition of thermotolerance (TD_{50} of 31 and 33 min for CPZ and TFP respectively). On the other hand, adding the drug after incubation also resulted in TD_{50} of 31 min in the case of CPZ and 33 min in the case of TFP, indicating sensitization of thermotolerant cells to the heat challenge. However, when cells were treated with the drug after heat shock to inhibit the thermotolerance, no further sensitization was observed.

It is thus evident that thermotolerance could be inhibited when the drug was present during incubation at 37°C. This could be ascribed to inhibition of protein (HSP) synthesis. In addition, the phenothiazine derivatives, when present during the heat challenge (50°C), caused significant thermosensitization of normal as well as thermotolerant cells.

Since the magnitudes of drug-induced thermal sensitiza-

tion of control cells (32 min vs 15–16 min) and heat-shocked cells (68 min vs 31–33 min) were almost identical (Table 1), it can be inferred that phenothiazines sensitize cells to heat and inhibit development of thermotolerance but by independent mechanisms. As cell membranes play a critical role in determining the hyperthermic sensitivity of cells in general^{8,9}, the structural alterations brought about in the membrane by phenothiazines¹⁰ could lead to thermal sensitization. Phenothiazines, like other inhibitors of protein synthesis, also overcome development of thermotolerance by virtue of their effect on protein synthesis. Hence, because of their thermosensitizing properties, the use of phenothiazines in combination with hyperthermia would be more beneficial than the use of inhibitors of protein (HSP) synthesis⁵ alone, such as cycloheximide.

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