EFFECT OF CYCLOHEXIMIDE ON NONPERMISSIVE TEMPERATURE KILLING IN tsH1 MUTANT CELLS

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Abstract—1. We investigated the mechanism of cycloheximide-induced heat protection. We proposed a hypothesis to account for the mechanism [Lee and Dewey (1986) Radiat. Res. 106, 98-110].
2. Cycloheximide protects cells from hyperthermic killing by means of protecting thermolabile proteins from denaturation.
3. For this study, we have employed temperature-sensitive mutant tsH1 which contains a thermolabile leucyl-tRNA synthetase.
4. By 15 h of incubation at the nonpermissive temperature of 39.5 or 40°C, 40 or 93% of mutant cells respectively, were killed. In contrast, wild type SC cells did not lose viability after this same incubation.
5. Although killing of tsH1 by incubation at the nonpermissive temperatures was mainly due to denaturation of a thermolabile leucyl-tRNA synthetase, cycloheximide did not protect mutant cells from killing. However, tsH1 and SC cells exhibited similar sensitivities to killing at 43°C and above. Furthermore, cycloheximide protected both cell types from hyperthermic killing.
6. There was a 200- or 700-fold increase in survival after 2.5 h at 43°C by treatment with cycloheximide in tsH1 or SC cell type, respectively. Thus, the cellular target(s) for hyperthermic killing at this temperature apparently are similar in both types of cells.
7. The data indicate that the mechanism behind cycloheximide-induced heat protection is probably not the prevention of protein denaturation.

Key Word Index: Cycloheximide; temperature-sensitive mutant; hyperthermic killing

INTRODUCTION

Many studies have shown that cycloheximide (CHM) treatment offers a great deal of protection from heat killing of mammalian cells (Palzer and Heidelberger, 1973; Lin et al., 1984; Lee and Dewey, 1986), although the mechanism of heat protection by this drug is still unknown. Lee and Dewey (1986) postulated that since thermal denaturation of proteins may be lethal events in the cells (Westra and Dewey, 1971; Massicotte-Nolan et al. 1981), cycloheximide may protect thermolabile proteins from denaturation. It is well known that glycerol, a heat protector, both stabilizes thermolabile proteins (Lepock et al., 1990) and protects cells from heat killing (Henle and Warters, 1982). Recent work from Hightower’s laboratory (Edington et al., 1989) showed that the presence of D2O or glycerol during a preheating that would normally induce stress proteins prevented heat shock protein (HSP) induction during a recovery period. Since the presence of abnormal proteins stimulates the synthesis of a set of stress proteins (Hightower, 1980; Goff and Goldberg, 1985; Ananthan et al., 1986; Lee and Dewey, 1987), this observation suggested that glycerol and D2O protected heat-sensitive proteins from thermal denaturation.

To examine if cycloheximide protects cells from hyperthermic killing by means of protecting thermo-labile proteins from denaturation, we have employed temperature-sensitive Chinese hamster ovary (CHO) mutant tsH1 cell. This mutant cell type contains a thermolabile leucyl-tRNA synthetase, which becomes inactive (denature at the nonpermissive temperature (> 34°C) (Thompson et al., 1973; Haars et al., 1976). Incubation of the cells above 34°C results in cell death (Haars et al., 1976). If CHM can protect the thermolabile leucyl-tRNA synthetase from denaturation, it should protect tsH1 mutant cells from the nonpermissive temperature killing.

MATERIALS AND METHODS

Cell culture and survival determination

The temperature-sensitive Chinese hamster ovary (CHO) mutant tsH1 cell line was originally isolated by Thompson et al. (1973). Exponentially growing mutant tsH1 (CHO-tsH1) and wild type (CHO-SC) cells were cultured in McCoy’s 5a medium (Cellgro) supplemented with 26 mM sodium bicarbonate and 10% iron-supplemented calf serum (HyClone). Three days prior to the experiment, 1.2 × 10⁶ cells were plated into T-75 flasks. The flasks containing cells were kept in a 34°C humidified incubator with a mixture of 95% air and 5% CO₂. For survival determination after various treatments, cells were trypsinized, counted and plated at appropriate dilutions. X-irradiated feeder cells (25 Gy) were used to maintain the plated cell density at 4000 cells/cm² (Highfield et al., 1984; Borrelli et al., 1989). After 1-2 weeks of incubation at 34°C, colonies were stained and counted.

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Drug treatment and drug toxicity

Cycloheximide (CHM, M.W. 281.3) was obtained from Sigma Chemical Co. Medium with drug was prepared 1 day prior to the experiment. Drug treatment was accomplished by aspirating the medium from the cells and replacing it with drug-containing medium. The drug treatment was terminated by aspirating the medium containing drug, rinsing twice with Hanks' balanced salt solution (HBSS) and replacement with drug free medium. CHM (10 μg/ml) treatment inhibited protein synthesis within 10 min by 95% (Lee and Dewey, 1986), but the drug caused minimum cytotoxicity when the duration of treatment was <17 h. For example, a CHM treatment of 17 h in SC or tsH1 cells decreased the plating efficiencies from 70 to 60% or from 72 to 63%, respectively.

Hyperthermic treatment

T-25 or T-75 flasks were heated by total immersion in a circulating water bath (Heto, Birkerod, Denmark), which was maintained within ±0.02°C of the desired temperature.

RESULTS

Effects of cycloheximide on survival at the elevated temperature in SC and tsH1 cells

Figures 1 and 2 show little or no killing at 39.5-40°C in wild type SC cells. However, temperature-sensitive mutant tsH1 cells lost viability at the nonpermissive temperatures. By 15 h of incubation at 39.5 or 40°C, 40 or 93% of mutant cells, respectively, were killed. As mentioned previously, this type of cell death was due to inactivation of a thermolabile leucyl-tRNA synthetase at the nonpermissive temperature (Haars et al., 1976). Nevertheless, treatment with CHM (10 μg/ml) did not protect tsH1 from killing at the nonpermissive temperature.

Figures 3 and 4 show that SC and tsH1 cells exhibited different sensitivities to killing at 41-41.5°C. Wild type cells developed thermotolerance which inferred from biphasic survival curves during chronic heating at 41-41.5°C. Furthermore, CHM added 2 h before and left on during heating at 41-41.5°C greatly protected SC cells from heat killing. There was an
Cycloheximide and nonpermissive temperature killing

Fig. 5. Effect of treatment with CHM (10 μg/ml) on cell survival at 43°C. Cells were treated with CHM for 2 h before and during incubation at 43°C (●, ▲). ○, △, Survival curve of untreated control cells. Left panel, survival curve of wild-type CHO-SC cell; right panel, survival curve of mutant CHO-tsH1 cell. Error bars represent 1 SD of the data for each point.

18-fold increase in survival from 0.05 to 0.9 after 6 h at 41.5°C (left panel in Fig. 4). In contrast, mutant type cells did not develop chronic thermotolerance and were protected by treatment with CHM to a much lesser degree, i.e. a 2.3-fold increase in survival from 0.04 to 0.09 after 6 h at 41.5°C (right panel in Fig. 4).

Unlike heating at 39.5–41.5°C, Fig. 5 shows that SC and tsH1 cells exhibited similar sensitivities to killing at 43°C. These results are consistent with observation from Chang et al. (1989). Furthermore, the large protective effects of CHM treatment on hyperthermic killing at 43°C were observed in both cell types. There was a 700- or 200-fold increase in survival after 2.5 h at 43°C by treatment with the drug in SC or tsH1 cells, respectively. In SC cells, the reciprocal of the slope (D0, time to reduce the survival in the exponential portion of the curve to 1/e) was 11 min for untreated cells, and 22 min for CHM-treated cells. In tsH1 cells, D0 was 12 min for untreated cells, and 22 min for CHM-treated cells.

DISCUSSION

Our experiments (Figs 1–5) demonstrate differences and similarities in the effects of CHM (10 μg/ml) on killing at elevated temperatures between temperature-sensitive mutant (CHO-tsH1) and wild type (CHO-SC) cells. Little or no protection by treatment with the drug was observed in mutant type cells when the cells were incubated at the nonpermissive temperatures of 39.5–41.5°C. In contrast, wild type cells became thermally resistant by treatment with CHM. Nevertheless, heat resistance to killing at 43°C was induced by treatment with CHM in both cells.

Vidair and Dewey (1989) have suggested that two modes of lethal events were involved in hyperthermic killing of tsH1 cells. One is the rapidly reversible type damage (Type I); the other is the slowly reversible type damage (Type II). Killing of tsH1 at nonpermissive temperature (39.5–40°C) may be mainly due to Type I death. This type of death is probably related to denaturation of thermolabile leucyl-tRNA synthetase (Haars et al., 1976). The inhibition of this enzyme activity was not thermostolerant (Vidair and Dewey, 1989) or was not protected by treatment with the heat protector, CHM, to low temperature hyperthermia in tsH1 cells (Figs 1–4). In contrast, at 43 or 45°C (high temperature hyperthermia or acute heating), the killing of mutant cells expressed thermostolerance (Vidair and Dewey, 1989) or was protected by CHM (Fig. 5). It suggests that the mode of death after acute heating in wild and mutant type cells is mainly due to Type II death. In other words, both cell types might have the identical cellular target(s) for acute heat killing at high temperatures.

Cycloheximide is well known as a heat protector (Palzer and Heidelberger, 1973; Lin et al., 1984; Lee and Dewey, 1986), although the mechanism of protection by the drug has not been well understood. Lee et al. (1987) clearly have shown that the mechanism of CHM-induced heat resistance does not involve the alteration of protein profiles, i.e. a selective synthesis of heat shock proteins. Furthermore, since Figs 1–4 clearly show that CHM does not protect thermolabile leucyl-tRNA synthetase from denaturation, our data suggest that the mechanism behind CHM-induced heat protection is not the prevention of protein denaturation. It remains possible, however, CHM only prevents the denaturation of a specific subset of intracellular proteins, or the disassembly of protein complexes. Obviously, further studies at the cellular and molecular levels are necessary to understand the mechanism of heat protection by CHM.

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REFERENCES


