GLUTATHIONE POOL SIZE AFFECTS CELL SURVIVAL AFTER HYPERThERMIC TREATMENT

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Intracellular glutathione (GSH) concentrations were titrated in Chinese hamster ovary cells by exposure to various concentrations of diethylmaleate (DEM). The various steady state levels of GSH obtained were maintained throughout the experimental time course. Cells were incubated at 42°C after DEM addition in order to produce thermal dose response curves using colony formation as the end point. The slope of the dose response curve was subsequently determined and compared to the intracellular GSH concentration. This comparison indicated Chinese hamster ovary cells contain multiple reservoirs of GSH which in turn regulate thermal toxicity in a stepwise manner. Removal of 50% or less of the GSH did not affect thermal sensitivity. A small increase in sensitivity occurred when 50 to 80% of the GSH was removed. Removal of greater than 80% of the GSH increased thermal toxicity significantly. The facts that 10 and 20 μM DEM produce extensive GSH depletion and only small changes in survival imply that a threshold concentration of GSH must be removed before thermal toxicity is affected.

INTRODUCTION

Therapeutic hyperthermia is the production of temperatures in excess of 41°C (usually between 42-45°C) in a tumor mass. The elevation of temperature can be accomplished by RF, ultrasound or microwave heating. If combined with either chemotherapeutic drugs or radiation, it will synergistically increase their effectiveness.

The target for hyperthermic induced cytotoxicity has yet to be identified. One target that has been postulated is loss of membrane integrity. This is based partly on the

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2. Key words: cytotoxicity, glutathione, hyperthermia, pool size.
3. Abbreviations: BCNU, 1-3-Bis(2-Chloroethyl)-1-nitrosourea; BSO, buthionine sulfoximine; CHO, Chinese hamster ovary; DEM, diethylmaleate; DMSO, dimethyl sulfoxide; HBSS, Hanks balanced salt solution; RF, radiofrequency.
observation of an increased rate of thermal inactivation in mutant K1060 E. Coli when linolenic acid was incorporated into membranes versus oleic acid (Yatvin, 1977). When L1210 cells incorporated docosahexaenoic acid (22:6) in membranes, a greater thermal sensitivity was exhibited compared to cells with high oleic acid membrane content (Guffy et al., 1982). Exposure to the anesthetic procaine has also been shown to increase hyperthermic cytotoxicity in E. Coli and L5178Y cells (Yatvin, 1977; Yau, 1979). Lepock et al. (1983) have compared the energy of thermal inactivation required for cell death with membrane lipid transitions, as determined by ESR, and with membrane protein transitions, as measured by intrinsic protein fluorescence. Their observations suggested that membrane lipid damage was not responsible for thermal cytotoxicity but that a correlation between membrane protein damage and toxicity may be significant.

Recently, it has been shown that glutathione (GSH) concentrations can modify hyperthermic toxicity. Depletion of GSH by either diethylmaleate (DEM) or buthionine sulfoximine (BSO) increased the rate of thermal inactivation in Chinese hamster ovary (CHO) and V-79 cells (Freeman et al., 1985; Mitchell et al., 1983). These observations combined with the reports that inhibition of superoxide dismutase by diethyldithiocarbamate (Lin et al., 1979). The increased thermal sensitivity suggests that GSH depletion could inhibit reduction of activated oxygen species and allow them to interact with the cellular target responsible for thermal inactivation.

Because GSH is important as a regulator of thermal sensitivity, it becomes important to know the concentration at which cytotoxicity is affected. The purpose of this present study was to determine the relationship between GSH pool size and the rate of thermal inactivation.

**METHODS**

CHO cells growing exponentially in monolayer cultures were maintained at 37°C and at pH 7.4 in McCoy's 5A medium supplemented with 10% fetal bovine serum, 2.2 g/l of sodium bicarbonate, 100 units/ml of penicillin G sodium, and 100 mg/ml of streptomycin sulfate. The cultures were found to be free of mycoplasma infection (Microbiological Associates, Bethesda, MD).

Survival experiments commenced by inoculating the appropriate number of test cells needed to yield 100-200 colonies in T-25 flasks (4 flasks/point) containing 4 ml of growth medium. In addition, 1 × 10⁴ lethally irradiated (2,000 rad) cells were inoculated into each flask. Experiments designed to measure GSH commenced by inoculating 5 × 10⁵ test cells per flask. The flasks were then incubated overnight at 37°C. All treatments occurred the following day.

DEM, > 99.9% pure, was dissolved in dimethylsulfoxide (DMSO). The DEM/DMSO solution was then diluted with glucose free Hanks balanced salt solution (HBSS) and then added to the flasks containing growth medium. Flasks not treated with DEM were