Received by

APR 24 1991

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ANL/CP--70911

DE91 010791

PROTECTION AGAINST UVA-INDUCED PHOTOOXIDATIVE DAMAGE IN MAMMALIAN CELL LINES EXPRESSING INCREASED LEVELS OF METALLOTHIONEIN

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INTRODUCTION

Metallothionein (MT) is an endogenous low molecular weight protein that is inducible in a variety of eukaryotic cells and has the ability to selectively bind heavy metal ions such as zinc and cadmium.¹ Although the exact physiological role of MT is still not understood, there is strong evidence that MT is involved in providing cellular resistance against the damaging effects of heavy metals and in the regulation of intracellular zinc and copper.^{2,3}

Recently, it has been demonstrated that MT can scavenge radiation-induced reactive oxygen intermediates *in vitro*,⁴ specifically hydroxyl and superoxide radicals, and because of these observations it has been suggested that MT may provide protection against radiation-induced oxidative stress *in vivo*. Cell lines expressing increased levels of MT have demonstrated resistance to ionizing radiation,⁵ to ultraviolet radiation,^{6,7} and also to various DNA damaging agents including melphalan and cis-diaminedichloroplatinum.⁸ It is therefore important to gain some insight into the relationship between cellular MT content and cellular resistance to radiation and other DNA damaging agents.

In this study we investigated the role of MT in providing protection against monochromatic 365-nm UVA radiation, which is known to generate intracellular reactive oxygen species that are involved in both DNA damage and cell killing.⁹ For this purpose, we used zinc acetate, a potent inducer of MT, to elevate MT levels in V79 Chinese hamster fibroblasts prior to UVA exposure and determined cell survival for uninduced and induced cultures. In order to eliminate any zinc effects other than MT induction, we also isolated and characterized cadmium chloride-resistant clones of V79 cells that have increased steady-state levels of both MT mRNA and protein, and we examined their survival characteristics against 365-nm radiation in the absence of zinc acetate.

METHODS

V79 cells were routinely cultured in RPMI 1640 media supplemented with 10% fetal bovine serum and grown under 8% CO₂ atmosphere at 37°C. MT was induced by treating cells with zinc acetate and quantitated (as moles of Cd bound per mg protein) using a method described previously.¹⁰

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METHODS

V79 cells were routinely cultured in RPMI 1640 media supplemented with 10% fetal bovine serum and grown under 8% CO₂ atmosphere at 37°C. MT was induced by treating cells in monolayer with 100 µM zinc acetate and quantitated (as nmoles of Cd bound per mg total protein) by the ¹⁰⁹Cd/hemoglobin affinity assay of Eaton and Toal.¹⁰ For exposure to monochromatic 365-nm UVA radiation, cells were treated with zinc acetate for 24 h, harvested, resuspended in phosphate buffered saline, and irradiated with stirring at room temperature according to Jones *et al.*¹¹ Control cultures were not treated with zinc acetate. Cell survival was measured by incorporation of ³H-hypoxanthine into total nucleic acid using the method of Cleaver and Thomas¹² and survival curves fitted to the linear-quadratic model using the least squares method. Clones of V79 cells containing elevated levels of MT were obtained by selecting for increased resistance to cadmium chloride toxicity. Briefly, V79

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cells were seeded at high density in media containing 20 μM cadmium chloride; these cells gave rise to the V79Cd1 clone, which subsequently produced V79Cd2 and V79Cd3 upon selection at higher cadmium chloride concentrations.

RESULTS

The induction kinetics of MT by zinc acetate in V79 cells and V79 clones over a 24 h time course is illustrated in Fig. 1. All four cell lines had similar induction profiles, although the V79 clones showed an increased constitutive level of MT, a reduced initial lag phase of induction, and achieve a higher level of MT by 24 h. V79 cells induced with zinc acetate had a 29-fold increase in cadmium-binding activity by 24 h when compared to control cultures. This time point of zinc acetate treatment was chosen to determine cell survival after 365-nm radiation exposures.

FIGURE 1. Kinetics of MT Induction by Zinc Acetate. V79 cells and V79 cadmium-resistant clones were exposed to 100 μM zinc acetate; cellular extracts were prepared and assayed for cadmium-binding activity. Activity is expressed as nmoles of cadmium bound per mg total protein.

Cell survival after 365-nm radiation was next determined for both uninduced and induced cultures of V79 cells in order to characterize any possible protection afforded by MT against UVA radiation-induced stress. Fig. 2 depicts the cell survival kinetics and provides evidence for the involvement of zinc acetate-induced MT in protection against UVA radiation. There is a significant increase in survival over the radiation dose range tested for the treated cells relative to the uninduced control cells. At a dose of 0.8 MJ/m^2 , the induced culture had 19% viable cells remaining, compared to 4% remaining in the uninduced culture.

There is current evidence from *in vivo* data that zinc deficiency causes lethal cellular damage by increasing lipid peroxidation of cell membranes and that supplemental zinc is able to stabilize membranes against such damage by acting either as a free radical

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FIGURE 2. 365-nm Survival Curves of Control and Zinc Acetate-Induced V79 Cells. *Cell cultures were harvested, resuspended in phosphate buffered saline at a cell density of 2×10^6 cells/ml, and exposed to 365-nm radiation.*

FIGURE 3. 365-nm Cell Survival Curves of V79 Cadmium-Resistant Clones. *V79 clones containing elevated MT levels were irradiated with 365-nm radiation without prior exposure to zinc acetate.*

scavenger or by reducing intracellular free radical formation.¹³ In order to eliminate any possibility that zinc alone confers protection against UVA-induced photooxidative damage, we isolated V79 cells with varying steady-state levels of both MT mRNA and protein by selecting for resistance against cadmium chloride and determined their survival against 365-nm radiation in the absence of zinc acetate. Figure 3 represents the survival curves of the V79 clones V79Cd1, Cd2, and Cd3 against 365-nm radiation with no zinc present. All three clones exhibited increased survival comparable to the zinc acetate-induced V79 cultures. Cell survival at 0.8 MJ/m² for uninduced cultures of V79Cd1, Cd2, and Cd3 was 14%, 20%, and 19%, respectively. These results tend to support the hypothesis that the increased cellular MT levels protect cells against UVA radiation by scavenging free radicals and that zinc itself is not responsible for the observed protection.

DISCUSSION

Ultraviolet radiation is generally divided into three wavelength regions designated as UVC (290 nm and below), UVB (290 to 320 nm), and UVA (320 nm to visible light). There is now sufficient evidence to indicate that exposure to UVA radiation, a major component of sunlight, represents an important oxidative stress to mammalian cells through the intracellular generation of reactive oxygen intermediates. We have demonstrated that MT, a free radical scavenger, can ameliorate the cytotoxic effects of monochromatic 365-nm UVA radiation and suggest that this occurs by MT reducing cellular oxidative stress and photooxidative damage through its interaction with certain reactive oxygen species. The nature of the reactive oxygen intermediates produced during UVA radiation may be similar to those produced by other DNA damaging agents suggesting that MT may provide a general radical scavenging mechanism for specific cell types. The type of UVA-mediated photooxidative damage that MT protects against is not yet known, but it may involve protection at the level of DNA since it is well known that wavelengths in the UVA range can cause various types of DNA damage including single-strand breaks and DNA-protein crosslinks.¹⁴ We are presently investigating whether MT can protect cellular DNA from the damaging effects of UVA radiation.

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ACKNOWLEDGEMENTS

This work was supported in part by PHS Grants RO1-CA34492 and RO1 CA37848 awarded by the National Cancer Institute, U. S. Department of Health and Human Services, and in part by the U. S. Department of Energy, Office of Health and Environmental Research, under contract No. W-31-109-ENG-38. The authors thank David Nadziejka for his editorial review and Kay Bexson for her excellent secretarial assistance.