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Short communication

Inhibition of mitomycin C's aerobic toxicity by the seleno-organic antioxidant PZ-51*

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Summary. Mitomycin C (MMC) is a bioreductive alkylating agent that is capable of generating oxygen radicals. Porfiromycin (PM) is an analog to MMC that generates oxygen radicals at a significantly lower level than the parent compound. Under aerobic conditions, the toxicity of MMC to EMT6 cells is 2.5-fold that of PM, whereas hypoxically the two are equitoxic. In the present studies, the protective effect of PZ-51 in combination with NAC was assessed against the dose-dependent toxicity of either MMC or PM under both aerobic and hypoxic conditions. Aerobically, the PZ-51 and NAC combination inhibited the toxicity of MMC at concentrations of between 0.25 and 2 μM but had no effect on PM toxicity. Under hypoxic conditions, the PZ-51 and NAC combination had no effect on either MMC or PM toxicity. These findings support a role for oxygen radical generation in the aerobic toxicity of MMC at clinically relevant doses.

Introduction

MMC is a naturally occurring antibiotic that is active against a variety of animal [3] and human [1] tumors. This agent is preferentially toxic to hypoxic as opposed to aerobic tumor cells in vitro [9, 13] as well as in vivo [15, 16]. The molecular mechanism by which MMC acts requires bioactivation to a reactive intermediate that can either produce DNA intrastrand cross-linking [8] or, under aerobic conditions, generate reactive oxygen species [4, 11, 12]. Porfiromycin (PM), a methylated analog of MMC, generates significantly fewer reactive oxygen species on incubation with EMT6 mouse mammary carcinoma cell sonicates and is less toxic to aerobic cells [12].

PZ-51 (Ebelsen) is a novel seleno-organic compound that has been found to exhibit antioxidant activity [10]. This antioxidant activity has been shown to be thiol-dependent and peroxidase-like in nature [17]. PZ-51 has been shown to moderate the cytotoxicity of anticancer quinones in Ehrlich tumor cells and MCF-7 cells [5, 6]. We extended these studies on tumor cells [5, 6] and demonstrated that this interaction is oxygen-dependent in nature and extends over a range of MMC concentrations.

Materials and methods

PZ-51 was a gift of the Ciba-Geigy Corporation (Summit, N.J.). PM was kindly provided by Dr. A. C. Sartorelli (Director, Yale University Comprehensive Cancer Center). EMT6 cells were grown in 25-cm^2 Corning plastic culture flasks (aerobic assays) or in glass milk-dilution bottles (hypoxic assays) at 37° C as previously described [16]. The characteristics of this cell line have been described elsewhere in great detail [14]. EMT6 cells were pretreated with either control solvent (dimethylsulfoxide, DMSO), NAC, PZ-51, or PZ-51 and NAC for 1 h. They were washed twice with Dulbecco's phosphate-buffered saline (DPBS) and then treated with either MMC, PM, or control solvent (ethanol) for 1 h. Following drug exposure, the cells were washed twice with DPBS, trypsinized off the flasks, counted, and plated onto replicate plates. Colony formation was measured after 10 days and the surviving fractions were determined from these measurements.

Under hypoxic conditions, the glass milk-dilution bottles were fitted with rubber sleeves, and EMT6 cells were flushed with N_2 for 1 h prior to the beginning of the pretreatment regimen and were continuously maintained under N_2 throughout the pretreatment and drug exposure. Drugs were given by injection through the rubber sleeve so as to avoid disrupting the hypoxic state. Statistical analysis was performed using Student's *t*-test, with significance being attributed to a value of $P < 0.05$.

Results

The effect of PZ-51 and NAC, both separately and in combination, on MMC and PM aerobic toxicity to EMT6 cells at the LD_{50} (lethal dose for 50% of the cells) was tested and the results are shown in Table 1. Pretreatment

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Abbreviations: DMSO, dimethylsulfoxide; DPBS, Dulbecco's phosphate-buffered saline; MMC, mitomycin C; NAC, *N*-acetylcysteine; PM, porfiromycin; PZ-51, 2-phenyl-1,2-benzoisoselenazol-3(H)-one

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Table 1. Effect of PZ-51, NAC, and PZ-51 + NAC on the aerobic toxicity of MMC and PM at an LD_{50} dose

Drug[a]	Pretreatment[b]	% of control values
MMC	–	52 ±2.2 (n = 10)
MMC	NAC	47.4±8.4 (n = 5)
MMC	PZ-51	62.7±4.1 (n = 6)*
MMC	PZ-51 + NAC	76.5±2.6 (n = 9)*,**
PM	–	48.4±4.7 (n = 8)
PM	NAC	53.7±3.8 (n = 6)
PM	PZ-51	51.9±2.8 (n = 7)
PM	PZ-51 + NAC	47.8±3 (n = 8)

[a] MMC, 1 μM; PM, 2.5 μM

[b] NAC, 1 mM; PZ-51, 50 μM

* Significantly different (P <0.05) from the nonpretreated group as determined using Student's t-test; ** Significantly different (P <0.05) from the PZ-51-pretreated group as determined using Student's t-test

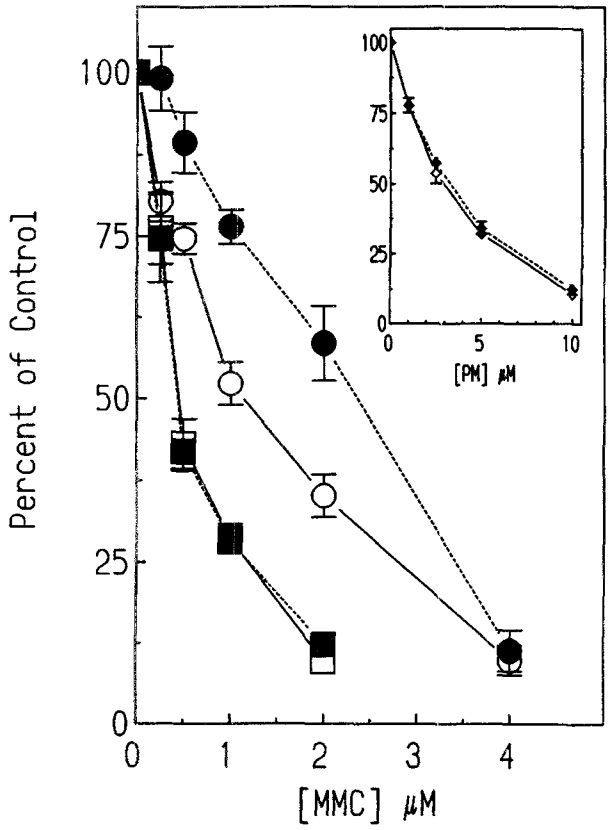

Fig. 1. Survival of EMT6 cells treated with MMC in the presence (*filled symbols*) or absence (*open symbols*) of PZ-51 (50 μM) and NAC (1 mM) pretreatment. *Circles*, Aerobic; *squares*, hypoxic. *Inset:* Effect of PZ-51 and NAC pretreatment on PM toxicity aerobically. *Filled symbols*, pretreated; *open symbols*, controls. Data represent the average of at least 3 independent experiments; bars indicate the SE

with PZ-51 alone or in combination with NAC prior to MMC exposure resulted in significant increases in cell survival. Pretreatment with NAC alone had no effect. PM toxicity was not significantly affected by pretreatment with either PZ-51 or the combination of PZ-51 and NAC.

The effects of the combination of PZ-51 and NAC were also tested using varying concentrations of MMC and PM under both aerobic and hypoxic conditions, and the dose-response curves obtained are shown in Fig. 1. The PZ-51 and NAC combination had a significant effect aerobically at doses ranging from 0.25 to 2 μM. At an MMC concentration of 4 μM, which approximately corresponds to the LD_{90}, the PZ-51 and NAC pretreatment had no effect on MMC-induced cytotoxicity. Under hypoxic conditions, the pretreatment had no effect at the four MMC concentrations tested. Pretreatment with the PZ-51 and NAC combination had no significant effect on PM-induced toxicity either aerobically (Fig. 1, inset) or hypoxically (data not shown).

Discussion

Mitomycin C is an antineoplastic agent that on bioactivation can generate oxygen radicals [4, 11, 12]. However, the role of these reactive oxygen species in MMC's antitumor activity remains unclear. MMC's activity in hypoxic systems [9, 13] suggests that oxygen radicals are not required for the drug to be toxic. MMC's toxicity to hypoxic tumor cells both in vitro and in vivo is well documented [9, 13, 15, 16], and alkylation of DNA or other macromolecules by the activated MMC molecule most likely represents the toxic event in these hypoxic systems. However, under aerobic conditions, oxygen may compete for this activated MMC molecule, giving rise to toxic oxygen radicals.

PZ-51's antioxidant activity is thiol-dependent and glutathione peroxidase-like in nature [17]. It is capable of detoxifying hydrogen peroxide as well as lipid hydroperoxides. PZ-51 has been shown to decrease MMC toxicity to Ehrlich tumor cells [5] and to protect MCF-7 tumor cells from doxorubicin-induced toxicity [6]. When used in combination with PZ-51 NAC, enhances the reduction of diquat-induced toxicity in isolated hepatocytes [2].

The mechanism by which PZ-51 reacts with hydroperoxides has been postulated to involve the thiol-dependent formation of a reactive diselenide [7]. The enhanced protection afforded by NAC in these studies could involve an increased uptake by the cells of the diselenide that formed extracellularly during the reaction of PZ-51 with the NAC. Reverse-phase high-pressure liquid chromatographic (HPLC) studies suggest that the diselenide is more hydrophobic than is PZ-51 [7] and should therefore enter cells more quickly than the latter. Since MMC can generate oxygen radicals and PM does so only at high doses and in small amounts, these results support the hypothesis that the mechanism of protection for PZ-51 under aerobic conditions involves the detoxification of reactive oxygen species generated by the interaction of bioactivated MMC with molecular oxygen.

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