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DNA RADIATION DAMAGE AND ITS MODIFICATION BY METALLOTHIONEIN

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Thiol compounds have long been known to protect living cells against the harmful effects of ionizing radiation. Maetallothionein is a naturally occurring low molecular weight polypeptide rich in cysteine residues and may be useful in protection against low-level radiation effects.

Radiation damage to DNA and its nucleotide components and the radioprotective effect of metallothionein have been studied in model chemical systems and compared to its effect on cells. Metallothionein acts both as a free radical scavenger and a reductant, and its radioprotective effectiveness has been studied as a function of dose, drug concentration, and in the presence and absence of oxygen. It is more effective in protecting against sugar-phosphate damage under hypoxic conditions. The chemical modification is greater than that of cell killing as measured by the loss of colony-forming ability. Dose reduction factors greater than two are observed for DNA radioprotection, but the values in cells are much lower. These findings will be discussed in terms of the molecular mechanisms and their implications.

KEY WORDS: DNA damage, Pi release, metallothionein, protection, ·OH radical, H-donation.

INTRODUCTION

Ionizing radiation produces alterations in critical cell targets, some of which result in deleterious biological effects.[1,2] Mammalian cells are two-thirds water,[3] and deposition of energy in the aqueous component leads to the ionization of water molecules and the subsequent formation of reactive water radiolysis species.[2,4] The majority of cellular radiation damage occurs as a result of reactions between these free radical species and target macromolecules, and is referred to as the 'indirect effect'.[4] The most important damaging species is the hydroxyl free radical (·OH),[5,6] an oxidant and electrophile that catalyzes or promotes the irreversible oxidation of cell targets.[7]

DNA is an important target for radiation damage to mammalian cells.[8,9] The free radical-mediated radiation damage occurring at the time of exposure,[10,11] if allowed to accumulate, unrepaired, may lead to chromosomal aberrations, mutations or other genetic defects resulting in acute or chronic biological consequences including cell killing and cancer.[7,12] Although radiation produces a broad spectrum of DNA damage, it can be classified as either base- or sugar-phosphate backbone damage.

There is good experimental evidence to support the fact that unrepaired double-strand breaks are an important lesion responsible for proliferative cell death.[12,13] The sparing effect of low dose-rates, the shoulder region on cell survival curves and split-dose experiments, and the linear-quadratic relationship for cell survival and DNA double-strand breakage all provide indirect supporting evidence.[8,9,12–14] Model

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chemical studies show that for low LET radiation double-strand breaks may arise from the accumulation of two single-strand breaks on adjacent strands of the DNA double helix.[9–13] These strand breaks are produced by direct ionization or by ·OH-induced H-abstraction[15] from the sugar moiety on the DNA backbone followed by hydrolytic cleavage of the sugar-phosphate bond.

The oxidative reactions involved are free radical mediated and are modifiable by either exogenous or endogenous redox chemicals.[7,16] These chemical additives alter the radiosensitivity of cells[17] and are known as radiation modifiers. Generally, reductants (red) act as radioprotectors and oxidizing agents (ox) are radiosensitizers. Protectors and sensitizers compete with each other for target radicals ($T^{\cdot}$) according to a redox model[7] which represents an extension of the earlier oxygen-fixation hypothesis:[18–20]

$$H_2O \sim\!\!\!\!\!\!\rightarrow (H_2O^*, H_2O^+ + e^-) \longrightarrow e^-_{aq}, H^{\cdot}, {}^{\cdot}OH \quad (1)$$

$$TH + {}^{\cdot}OH \longrightarrow T^{\cdot} + H_2O \longrightarrow \text{permanent oxidative target damage} \quad (2)$$

$$\text{protection } TH \xleftarrow{\text{red}} T^{\cdot} \xrightarrow{\text{ox}} T_x \text{ sensitization} \quad (3)$$

Sulphydryl agents including cysteine, cysteamine and glutathione, like vitamins C and E and other antioxidants when present during irradiation, protect mammalian cells against cell lethality[21] and against DNA strand breaks,[22] and experiments in model systems[7,15,16] support and quantify the extent and chemical kinetics of the above molecular mechanism.[7,12,18–20,23]

Exogenous thiols may undergo redox reactions with cells prior to irradiation, reducing or eliminating their potential radiobiological effectiveness.[21] This reactivity may also produce unwanted cycotoxicity.[21] In an attempt to circumvent these possible drawbacks to the development of an effective chemical radioprotector, a natural, thiol-containing, inducible protein, metallothionein, has been tested and its underlying molecular mechanism explored.

MATERIALS AND METHODS

Horse kidney (Cd, Zn) metallothionein-1 was obtained from Sigma Chemical Co. and used directly in aqueous solution or phosphate buffered saline. V-79 Chinese hamster cells from log phase cultures were irradiated in triplicate in the presence and absence of metallothionein using a Siemens Stabilipan 250 kV X-ray source at a dose-rate of $\sim 4\,Gy\,m^{-1}$. Metallothionein was administered 18 h prior to irradiation. Cells were maintained in Sigma DEM-F12 medium supplemented with 15% fetal bovine serum, incubated at 37.5°C in an atmosphere of 5% CO_2 in air for 1 week, after which time the resulting colonies were stained and scored to provide data for cell survival curves.[23]

Aqueous solutions of deoxyadenosine-5′-monophosphate ($10^{-3}\,mol\,L^{-1}$) were irradiated in a Gamma cell 220 Cobalt 60 source (Atomic Energy of Canada Ltd.) under a variety of conditions, at a dose-rate of $1.2\,kGy\,h^{-1}$. Samples (1 mL) were irradiated after saturating with oxygen or nitrogen. Inorganic phosphate (Pi) release was determined colorimetrically[24] using a Technicon Autoanalyzer, and the yield of Pi release

expressed as a G-value (defined as the number of Pi molecules released per 100 eV of absorbed radiation dose), as obtained from the slopes of the linear yield-dose plots.

RESULTS

Various concentrations of metallothionein (MT) from 5 to 100 $\mu g\,mL^{-1}$ (~ 0.7 to $14 \times 10^{-6}\,mol\,L^{-1}$) were incubated with V-79 cells overnight and during irradiation and the surviving fraction scored and compared with control cells irradiated in the absence of MT. Typical survival curves are shown in Figure 1 for aerobic cells irradiated at various doses in the presence and absence of a fixed concentration of MT ($25\,\mu g\,mL^{-1}$). These cells show a rather modest radioprotective effect of MT. The dose reduction factor (drf) was 1.25. This concentration of MT appears to be the optimum, no significantly different protection being observed at higher concentrations up to $100\,\mu g\,mL^{-1}$. This result is in contrast to that for cysteamine where an equivalent thiol concentration produced a drf $\sim$ 2 under aerobic conditions.

The yields of Pi released from deoxyadenosine-5′-monophosphate (5′-dAMP) increase linearly with dose giving values of 0.20 and 0.38 in deoxygenated and oxygen-saturated solution, respectively. The radioprotective effect of adding MT ($25\,\mu g\,mL^{-1}$) and cysteine ($10^{-4}\,mol\,L^{-1}$) to deoxygenated solutions of 5′-dAMP is shown in Figure 2. Dose reduction factors of 2.2 and 2.5 are observed for cysteine and MT under these conditions. A comparison of the data obtained in the presence and absence of oxygen is given in Table I. The radioprotective effect of these two thiol compounds in oxygen-free solutions is almost completely eliminated in the presence of oxygen.

DISCUSSION

The data in Figure 1 show that the *in vitro* radioprotective effectiveness of MT is smaller than expected on the basis of the model chemical studies. The radioprotection appears to be largely due to a slope- rather than shoulder-modification, indicative of a radiation-chemical action. It has been shown in separate experiments that MT at concentrations up to $100\,\mu g\,mL^{-1}$ produces no appreciable cytotoxicity following overnight incubation, suggesting that chemical reactivity with endogenous redox equivalents is not a significant factor in determining the potential usefulness of MT as a radioprotective agent. An unexpected observation is that MT appears to protect against cell killing under aerobic conditions. This is contrary to the protective effect of MT on DNA backbone damage which is only expressed predominantly in the absence of oxygen as expected by the oxygen-fixation hypothesis.[18–20]

Several explanations are possible for the protective effect of MT against cell killing in air. It may be that the protection in hypoxia is reduced by problems associated with drug delivery, uptake or metabolism. An oxygen-dependent drug transport process would explain such a differential effect. Alternatively, a biochemical[23,25] effect may be involved, but this is not consistent with the observed slope modification and unaltered shoulder region. However, some form of free radical-mediated thiol-catalyzed chain reaction leading to increased oxygen consumption could be involved, although this effect would be expected to increase with increasing MT concentration and no such

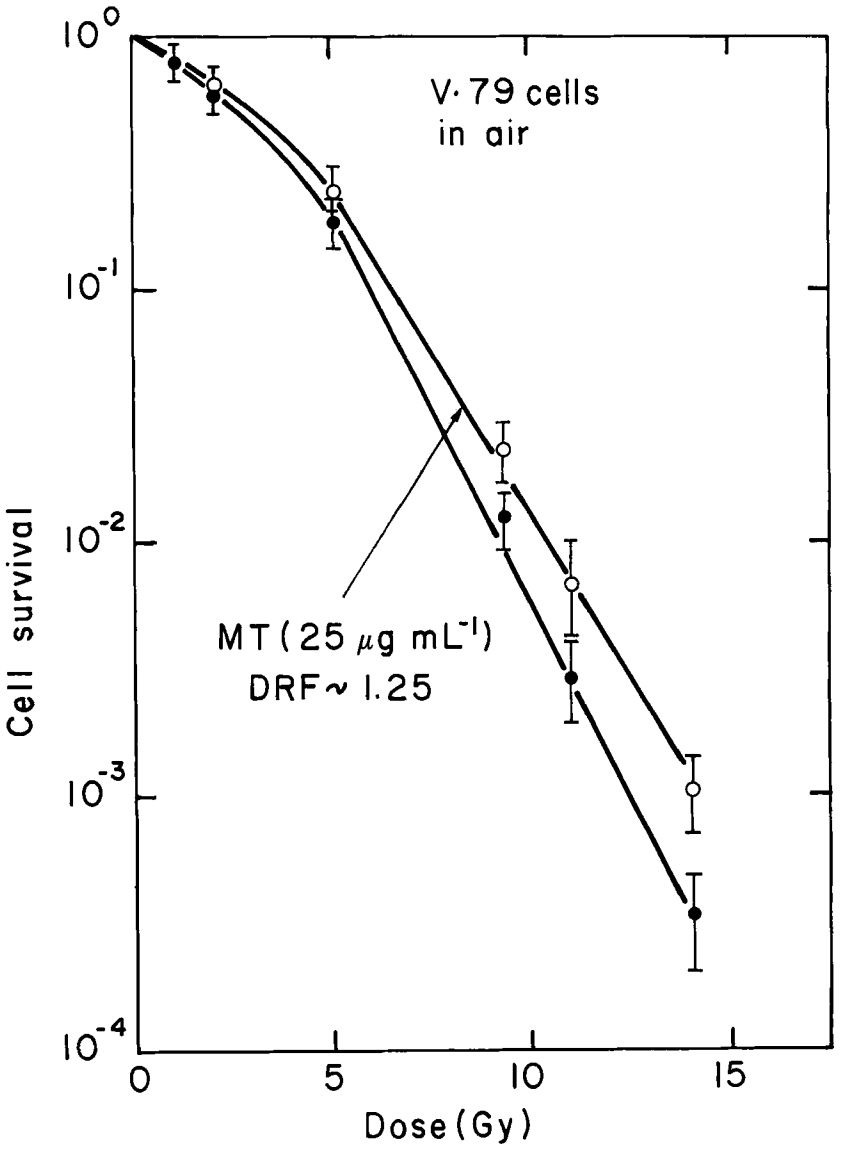

FIGURE 1 Survival curves for V-79 Chinese hamster cells irradiated in air with 250 kV X-rays in the presence (○) and absence (●) of MT, showing the modest radioprotective effect of metallothionein (MT).

increase is observed. The role of chelated Cd and Zn may also play a role. Whatever the explanation, a radioprotective drug selective for aerobic tissue would be useful for low-level radiation protection of exposed personnel, and normal tissue protection in radiotherapy patients. Metallothionein is a low molecular weight (6000–7000), thiol-containing, intracellular protein (up to 30% -SH) which is inducible in mammalian

TABLE I
Sugar-phosphate damage to deoxyadenosine-5′-monophosphate (5′-dAMP) as measured by inorganic phosphate (Pi) release, and the effect of cysteine and metallothionein (MT)

Chemical system	Radiation yield	
	$G(Pi)_{N2}$	$G(Pi)_{O2}$
5′-dAMP alone (10^{-3} mol L^{-1})	0.20	0.38
5′-dAMP with cysteine (10^{-4} mol L^{-1})	0.09	0.35
5′-dAMP with MT (25 μg mL^{-1})	0.08	0.36

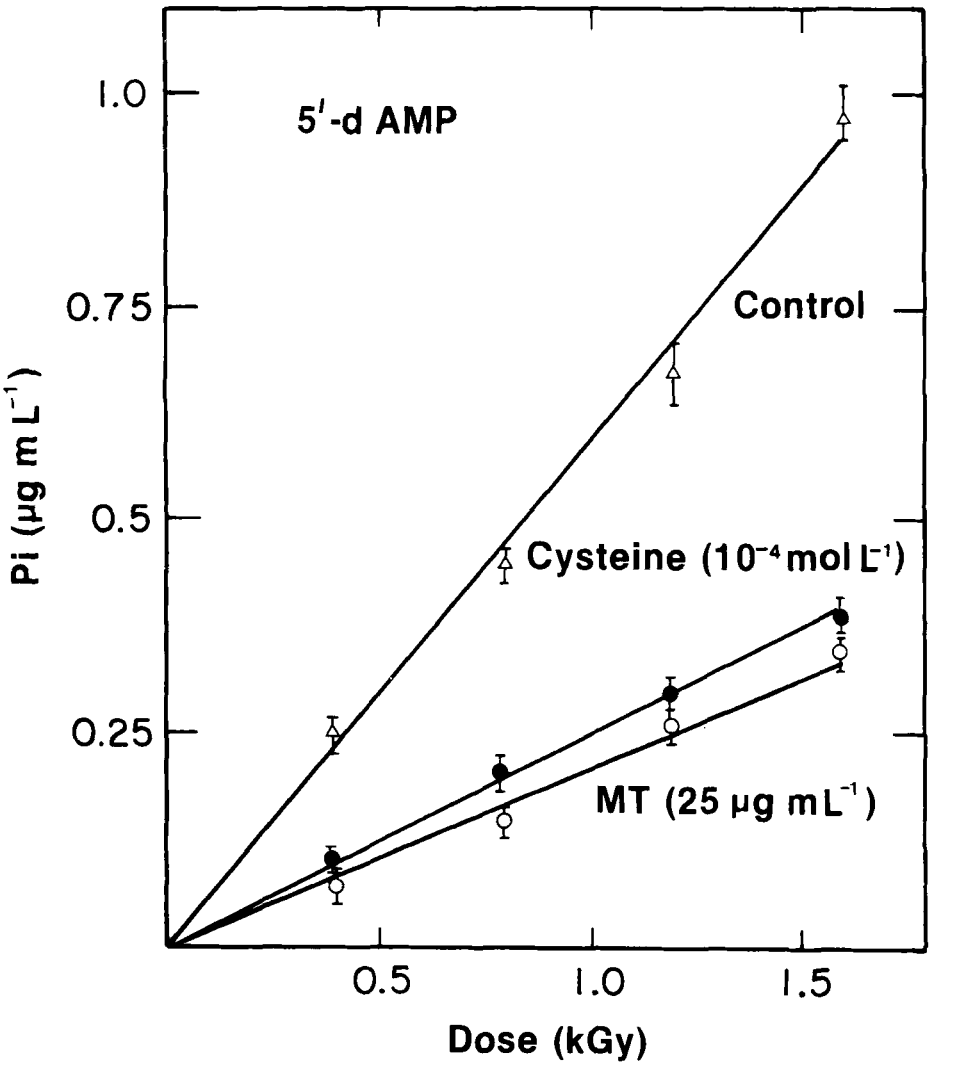

FIGURE 2 Effect of cysteine and metallothionein (MT) on the yield of radiolytic inorganic phosphate (Pi) release from deoxygenated solutions of deoxyadenosine-5′-monophosphate (5′-dAMP) (10^{-3} mol L^{-1}). From the slopes of the yield-dose plots, dose reduction factors of 2.2 and 2.5 are calculated for cysteine and MT respectively.

tissues as a defence against the oxidative threat posed by Cd, Zn and other toxic metals.[26] In view of its action as an antioxidant, it has the potential of being a non-toxic, natural radioprotector with minimal side-effects, used on an exogenous basis.

The radiation chemical studies clearly show that (Zn, Cd) metallothionein-1 is an effective radioprotector of DNA sugar-phosphate backbone damage as modelled by Pi release from the irradiated mononucleotide 5′-dAMP. In fact, on a molecular basis, MT is about five times more effective than cysteine. Since MT has 20-SH groups and 7 metal ions,[26] this high radioprotective efficacy is not unexpected. It is interesting to speculate whether the total removal of Zn and Cd ions will produce a further increase in radioprotective effectiveness.

The 2 1/2-fold protection of 5′-dAMP (10^{-3} mol L^{-1}) by 3.5×10^{-6} mol L^{-1} MT suggests that ·OH-scavenging is probably not the main mechanism. The rate constant for the reaction of ·OH with 5′-dAMP is $\sim 5 \times 10^{9}$ mol L^{-1} s^{-1},[27] and for MT to compete effectively it would require an ·OH-reactivity $\sim 1.5 \times 10^{12}$ mol L^{-1} s^{-1} which is higher than that for other proteins[27] containing, as MT does, no aromatic amino acids.[26] If ·OH-scavenging were the principal mode of action, a similar effect would be expected in oxygenated solution. The lack of chemical radioprotection in the presence of oxygen supports the hypothesis[7,15–20] outlined in reactions 2 and 3, that thiols donate an H-atom to a cellular target radical (in this case, a radical on the sugar or the base moiety of 5′-dAMP), thus restoring 5′-dAMP to its undamaged state:

$$5'\text{-dAMP} + {}^{\cdot}\text{OH} \underset{\text{MT}}{\rightleftharpoons} 5'\text{-dAMP sugar radical} \xrightarrow[O_2]{} \text{Pi release} \qquad (4)$$

Metallothionein competes with oxygen for the 5′-dAMP sugar radical, and is therefore less effective as a chemical radioprotector in the presence of oxygen.

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