# AMIFOSTINE: MECHANISMS OF ACTION UNDERLYING CYTOPROTECTION AND CHEMOPREVENTION

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**SUMMARY**

Amifostine is an important drug in the new field of cytoprotection. It was developed by the Antiradiation Drug Development Program of the US Army Medical Research and Development Command as a radioprotective compound and was the first drug from that Program to be approved for clinical use in the protection of dose limiting normal tissues in patients against the damaging effects of radiation and chemotherapy. Its unique polyamine-like structure and attached sulfhydryl group give it the potential to participate in a range of cellular processes that make it an exciting candidate for use in both cytoprotection and chemoprevention. Amifostine protects against the DNA damaging effects of ionizing radiation and chemotherapy drug associated reactive species. It possesses anti-mutagenic and anti-carcinogenic properties. At the molecular level, it has been demonstrated to affect redox sensitive transcription factors, gene expression, chromatin stability, and enzymatic activity. At the cellular level it has important effects on growth and cell cycle progression. This review focuses on relating its unique chemical design to mechanisms of action that underlie its broad usefulness as both a cytoprotective and chemopreventive agent for use in cancer therapy.

**KEY WORDS**

amifostine, cytoprotection, chemoprevention, mechanisms

## 1. INTRODUCTION

Amifostine (S-2-[3-aminopropylamino]ethylphosphorothioic acid), also referred to as WR-2721, ethiophos, and Ethyol®, has been approved for limited clinical use by the United States Food and Drug Administration as a cytoprotector to reduce associated dose limiting toxicities of cisplatin and ionizing radiation to normal tissues. Preclinical and clinical studies support the use of amifostine to protect against cancer therapy-induced neutropenia, nephrotoxicity, ototoxicity, neurotoxicity, mucositis, and xerostomia in patients /1-3/. Interest is also fostered by exciting new information describing diverse and novel medical applications of this drug. These range from affecting multilineage hematological effects and the process of hematopoiesis

/4,5/, to preventing cancer therapy-induced mutagenic and carcinogenic damage to normal cells /6-9/. Over 1,000 manuscripts have been published to date regarding the preclinical and clinical properties of amifostine and its thiol (WR-1065) and disulfide (WR-33278) forms. Several excellent reviews have been written regarding amifostine's development and potential clinical usefulness /10-14/. For this reason, the present review does not focus on either the current clinical status of amifostine or on cataloguing the myriad of potential novel applications arising out of preclinical and clinical research. Rather, it focuses on amifostine itself. By understanding the rationale underlying its design and resultant molecular structure, mechanisms of action can be readily identified and characterized for this interesting and multifaceted drug.

## 2. RATIONALE FOR DRUG DESIGN OF AMIFOSTINE

With the advent of the nuclear age, and driven by fears emanating from the Cold War, a drug development program was initiated by the United States Government for the purpose of developing chemical agents that could afford protection to people against the lethal effects of ionizing radiation. While the Antiradiation Drug Development Program was initiated by the US Army in 1959 and resulted in the synthesis and characterization of over 4,000 radioprotective drugs by 1979 /15/, the first demonstration of a chemical agent capable of protecting cells from radiation-induced lethality was reported ten years earlier /16/. The sulfhydryl-containing amino acid cysteine was demonstrated to be effective in protecting against the lethal effects of ionizing radiation. From this seminal study a number of conclusions were generated that formed the basis for subsequent investigations to develop effective radiation protective drugs. First, radiation protection can be achieved with a drug having both positively charged amine groups and a free sulfhydryl, i.e. an aminothiol. Second, the disulfide form of the drug is ineffective in conferring protection to cells exposed to ionizing radiation. Third, the aminothiol must be administered before and be present during the exposure of cells to ionizing radiation. And fourth, the magnitude of protection is proportional to the amount of the drug administered. An excellent review of this early literature is found elsewhere /17/.

In retrospect, the development of amifostine represents an example of what now is termed "rational drug design". The generally accepted target for cell killing by ionizing radiation was and still is considered to be the nuclear DNA of cells. The two mechanisms by which ionizing radiation can damage DNA are through the direct deposition of energy and subsequent ionization of atoms comprising the DNA, i.e. a direct effect, and the excitation and ionization of other important cellular molecules, such as cellular water, resulting in the generation of highly reactive free radicals, i.e. an indirect effect. The direct effect accounts for about 25% of the damage while the remaining 75% is due to highly reactive free radical-induced damage. The direct effect cannot be protected against using chemical agents. For this reason, research into the development of chemical radioprotectors has focused on reducing indirect damage to the DNA caused by highly reactive free radicals. The process of free radical formation is quite rapid. Radiolytic products are produced within $10^{-12}$ seconds following exposure to ionizing radiation. By $10^{-9}$ seconds following irradiation, free hydroxyl radicals are produced in high concentrations, and within the microenvironment of DNA will react readily with the DNA to induce damage. DNA damage by radiation-induced free radicals is basically completed by $10^{-3}$ seconds /18/. Thus to be protective against radiation-induced oxidative damage, the chemical radioprotector must be present at the time of radiation exposure.

Having identified both the cellular target and the mechanism of action for radiation-induced damage, it follows that the desirable properties of any effective chemical protector would be its ability to scavenge free radicals while being readily localized within the microenvironment of the DNA. Amifostine, an aminothiol, meets these criteria (see Fig. 1). As in the case of naturally occurring polyamines, the presence of positively charged amine groups facilitates the electrostatic binding of these molecules to negatively charged regions of DNA /19,20/. The presence of a sulfhydryl group enhances the free radical scavenging ability within the microenvironment of the target DNA. It was proposed as early as 1967 that aminothiols possessed polyamine-like properties that allowed them to bind to and stabilize any part of the DNA helix that was not covered by histones /21/. In this manner the damaged ends of DNA strands could be stabilized during repair thus allowing for a more effective repair. DNA replication would also be delayed, thus increasing the probability that

**Amifostine (WR-2721)**

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}PO_3H_2$

+

**Alkaline Phosphatase**

⇓

**WR-1065**

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}SH$

**Reduction** ⇑ **Oxidation** ⇓

**WR-33278**

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}S\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$

**Spermine**

**Fig. 1:** Relationship between amifostine and its thiol and disulfide forms and the polyamine spermine.

repair would be completed before the damaged regions could be replicated or passed on to daughter cells following mitosis. This model suggests that aminothiols can participate in two general processes: the reduction of initial damage to DNA induced by ionizing radiation by virtue of the radical scavenging ability of the free thiol form, and the enhancement of post-irradiation repair processes through the polyamine-like structure and function of the disulfide form by stabilizing the genome and delaying DNA replication.

The most significant development from the Drug Synthesis and Development Program was the synthesis and early testing of the phosphorothioates. These are aminothiols in which the sulfhydryl is

shielded by a phosphate group, resulting in a less toxic but ineffective radioprotector. This class of drug is a prodrug that requires dephosphorylation by alkaline phosphatase in order to be activated /22,23/. Following the formation of the active thiol form the molecule can interact with other thiols to form either its symmetrical disulfide or mixed disulfides. As shown in Figure 1, the symmetrical disulfide form (i.e., WR-33278) of amifostine and the polyamine spermine are similar in structure.

Amifostine emerged as the most effective radioprotector from the Drug Synthesis and Development Program. Several factors diminished its, as well as other chemical protectants' utility for use in military applications. First, to achieve the stated goal of affording a radioprotection enhancement factor of 2, individuals would require the administration of relatively high doses of the drug that also caused severe side effects, such as nausea, vomiting, and hypotension. In addition, to achieve the high concentrations of amifostine required for this level of radiation protection, the only effective route of administration is by intravenous injection. Finally, to achieve optimum effectiveness, amifostine must be administered 30 min before a potential radiation exposure /24/. While these requirements limited its usefulness for military operations, they are readily achievable in a medical setting. This, coupled with the observations of Yuhas *et al.* /25/ that amifostine could differentially protect normal tissues as compared to malignant tissues, has led to the clinical research, development, and implementation of amifostine as a cytoprotective drug for use in cancer therapy. Thus, the application of amifostine for clinical use as a cytoprotector had its genesis in a radioprotector program that was conceived by and focused to meet the specific needs of the military to perform its mission on a nuclear battlefield.

## 3. DOES AMIFOSTINE DIFFERENTIALLY PROTECT NORMAL AS COMPARED TO MALIGNANT TISSUES?

The most controversial question regarding the clinical use of amifostine in cancer treatment is whether or not it differentially protects normal as compared to malignant tissues. To best address this question, amifostine should be evaluated in light of its military legacy, as well as its known mechanisms of action. The target of a protection

factor of 2 severely impacted on the identification of a suitable radioprotector for military use because it required extremely large and toxic doses of the radioprotective drugs to be administered. This is because the magnitude of protection that can be achieved is directly related to the concentration of the drug present at the time of exposure to radiation (see Fig. 2) /6/. Protection factors for various normal tissues and/or their associated function are summarized in Table 1. These early animal studies formed the basis for later clinical investigations of amifostine as a cytoprotector /25-34/.

As described in Table 1, most tissues were protected with a dose of 400 mg/kg or higher. In the mouse system these doses are very high

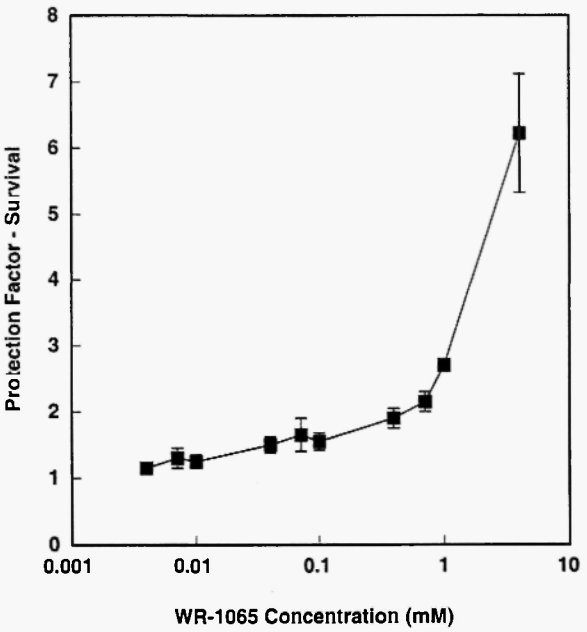


**Fig. 2:** Ratio of cell-surviving fractions for WR-1065 treated to untreated cells plotted as a function of WR-1065 concentration. Cells were exposed to a dose of 7.5 Gy from a $^{60}Co$ source. The WR-1065 treatment time was 30 min just before irradiation. All experimental points are the average of three separate experiments, and error bars represent the standard error of the mean (SEM). Reprinted from Grdina *et al.*[6] by permission of Oxford University Press.

**TABLE 1**

Survey of protection factors for amifostine against radiation-induced damage to different normal mouse tissues

| Tissue | WR-2721 dose (mg/kg) | Assay system | Protection factor | Ref. |
|---|---|---|---|---|
| **Bone marrow** | 200 | Survival of colony forming units | 2.3 | /25/ |
| | 200 | $LD_{50}$ | 1.8 | |
| **Lung** | 400 | $LD_{50}$ (pneumonitis) | 1.4 | /26/ |
| | 400 | Damage to Type II cells | 1.2 | |
| | 400 | Damage to endothelial cells | 1.2 | |
| **Kidney** | 400 | Tissue damage or kidney weight | 1.3 | /27/ |
| **Parotid gland** | 400 | Salivary flow rate | 2.9 | /28/ |
| **Oesophagus** | 400 | $LD_{50}$ (acute damage) | 1.6 | /29/ |
| | 400 | $LD_{50}$ (chronic damage) | 1.5 | |
| **Jejunum** | 400 | Number of regenerating crypts | 1.6-1.8 | /29,30/ |
| **Colon** | 400 | Survival of clonogenic epithelial crypt cells | 1.4-1.6 | /29/ |
| **Rectum** | 400 | Histologically observed damage | 1.3-1.4 | /29/ |
| **Spermatogonia** | 400 | Genetic damage | 2.4 | /31/ |
| | 400 | Survival of testicular stem cells | 1.3-1.4 | /32/ |
| **Skin** | 200-500 | Tissue damage | 1.2-1.6 | /33/ |
| **Hair follicles** | 400 | Hair loss in 50% of animals | 1.3 | /34/ |

and approach a toxic level for the mouse strains used in the studies. However, these doses were required to achieve protection factors approaching or reaching a factor of 2. While a protection factor of 2 is clearly relevant for useful military applications, the use of the relatively high doses of amifostine necessary to approach this level of protection for clinical applications has led to a number of problems. Rather than specifying acceptable levels of protection for various normal tissue endpoints that could afford utility in cancer treatment, the early clinical trials were based on the underlying goal of achieving the maximum protection factor possible. As an example, a dose of 910 $mg/m^2$ of amifostine was originally established for use in clinical chemotherapy studies. With this dose came a range of side effects including nausea, vomiting, hypotension and hypocalcemia /35/. The requirement of supportive care in the administration of amifostine to these patients limited its acceptance in many instances. With the recognition that lower doses of amifostine can afford clinical utility, clinical investigations are now being performed using amifostine doses as low as 200 $mg/m^2$ /36/. As the dose of amifostine is lowered, the severity of accompanying toxic side effects is also reduced or is no longer apparent. Thus, the attempt to translate the novel concept of cytoprotection by amifostine from a military to a medical application carried with it the perception that higher doses are better than lower, and that the ultimate goal to be achieved was the attainment of the highest protection factor possible. This perception led to the early clinical experience with amifostine that generated considerable concern regarding its dose-limiting toxic side effects.

Based on the early observations of Yuhas and Storer /37/, the premise underlying the development and application of amifostine as a cytoprotector for use in cancer therapy is that the drug preferentially protects normal as compared to tumor tissues. As new investigators entered the field to test this hypothesis, a number of reports began to appear suggesting that mouse tumors could be protected to similar levels previously achieved for their normal tissue counterparts (see Tables 1 and 2) /38-43/. Yuhas addressed these data and the issue of tumor protection in 1983 /44/. First, the preponderance of data suggests that solid tumors are either not or only marginally protected as compared to normal tissue end points. Second, tumor protection can be demonstrated for well oxygenated ascitic tumors or leukemias. The

**TABLE 2**

Survey of protection factors for amifostine against radiation-induced damage to mouse tumors

| Tumor | WR-2721 dose (mg/kg) | Assay system | Protection factor | Ref. |
|---|---|---|---|---|
| **Lewis lung carcinoma** | 500 | Cell survival | 1.3 | /38/ |
| **P388 leukemia** | 500 | Cell survival | 1.4 | /38/ |
| **FSa** | 400 | Micro-colonies Clonogenic assay | 1.2 | /39/ |
| **Fibrosarcoma** | 400-600 | Regrowth | 1.2-2.5 | /40/ |
| **Carcinoma** | 250-600 | Regrowth | 1.2-2.5 | /40/ |
| **RIF-1** | 400 | Clonogenic assay | 1-20 (variable) | /41/ |
| **FSa** | 400 | $TCD_{50}$ | 1.1 | /42/ |
| **FSa** | 400 | Latency | 1.3 | /42/ |
| **MCa-4** | 400 | $TCD_{50}$ | 1.3 | /42/ |
| **NFSa** | 400 | $TCD_{50}$ | 1.2 | /42/ |
| **RIF-1** | 400 | Clonogenic assay | 1.5-1.7 | /43/ |

implication of the latter conclusion is that well oxygenated metastatic disease could also be protected by amifostine /45/. Examples of amifostine cytoprotection against the therapeutic effects of cyclophosphamide can be demonstrated in experimental animal systems using the Lewis lung carcinoma /46/ and fibrosarcoma /47/ "artificial" pulmonary metastases models.

There is at present little evidence from the clinical data that amifostine protects advanced solid tumors from either chemo- or radiation therapy /3/. The elements of drug delivery, relative concentration of drug, and timing of administration are the prime mediators underlying the preferential protection of normal versus tumor tissues under *in vivo* conditions. As discussed earlier, cytoprotection by

amifostine is dependent upon its concentration in the target tissue at the time of radiation or chemotherapy exposure. Evidence also suggests that a threshold concentration must be surpassed in order to demonstrate any level of cytoprotection. In contrast to the regular and ordered nature of the vasculature supplying blood to normal tissues, the vasculature of tumors is best described as being highly abnormal consisting of distended and leaky capillaries that lead to impaired and relatively sluggish blood flow /48/. Thus drug delivery is significantly impaired in tumor as compared to normal tissue. The finding, however, that under experimental conditions some tumors can be protected demonstrates that low levels of amifostine can be achieved under certain conditions in some tumors /38-43/. A demonstration of drug uptake by tumors does not necessarily translate into tumor protection. As demonstrated in Figure 2 /6/, there is a threshold concentration of amifostine that must be surpassed in order to demonstrate cytoprotection. This is best described using the "artificial" pulmonary metastases model of Milas *et al.* /47/. Animals four days following the intravenous injection of known numbers of viable fibrosarcoma tumor cells develop "artificial" pulmonary metastasis that contain about 100-1000 cells that are well vascularized. Animals are exposed to amifostine or cyclophosphamide alone or in combination and the resultant effects on tumor growth are determined 14 to 21 days later. Using this system, it was demonstrated that an amifostine dose of 400 mg/kg was sufficient to protect fibrosarcoma micro-tumors from the therapeutic effects of doses of cyclophosphamide ranging from 20 to 100 mg/kg by a factor of 1.8 (see Fig. 3) /47/. However, if the concentration of amifostine was reduced to only 100 mg/kg while the cyclophosphamide dose remained at 100 mg/kg, no protective effect was observed (see Table 3) /49/. This lack of cytoprotection of fibrosarcoma micro-tumors growing in the lungs of mice by amifostine at a dose of 100 mg/kg extended across the entire dose range of cyclophosphamide used in the study by Milas *et al.* /47/ and is described in Figure 4 /50/.

The issue continues to remain open as to whether or not tumor cells, if exposed to high doses of amifostine, can be protected from the toxic effects of chemotherapeutic drugs and ionizing radiation. The most definitive *in vitro* study suggesting that tumor cells are not protected by WR-1065 was performed using fibrosarcoma cells

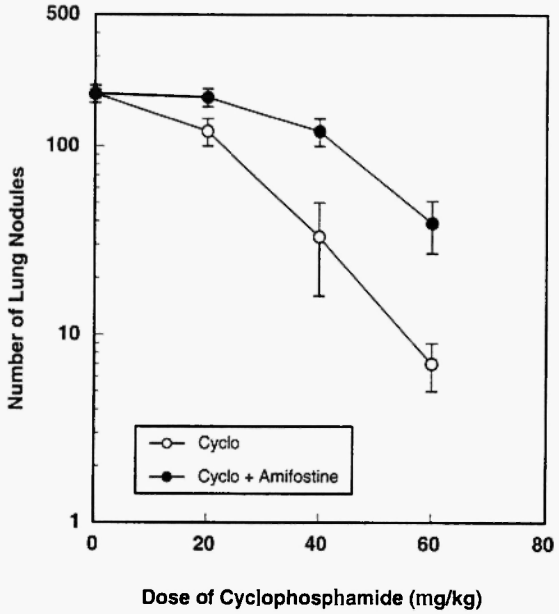


**Fig. 3:** Effect of amifostine on cyclophosphamide-induced killing of murine fibrosarcoma (FSA) tumor cells as evidenced by changes in the number of tumor nodules in the lungs of treated mice. Animals were given injections i.v. of 2 x $10^5$ viable FSA cells, and four days later, when tumor nodules in the lung were microscopic in size, animals were given i.p. graded doses of cyclophosphamide (cyclo) alone (O) or 400 mg/kg amifostine and cyclophosphamide (cyclo + amifostine) (●). Amifostine was administered 30 min before cyclophosphamide. Error bars represent SEM. Reprinted from Milas *et al* [47] by permission of the American Association for Cancer Research.

exposed to ionizing radiation and 4 mM WR-1065. No cytoprotection was demonstrated using a standard colony forming survival assay. In contrast, normal human diploid fibroblasts exposed under the same conditions were protected by a factor of 1.9 /51/. An attempt was made to demonstrate that WR-1065 was not protective against a broad range of standard anticancer drugs using human ovarian and breast cancer cells /52/. Unfortunately, this comprehensive study was flawed in a number of areas. First, tumor cells were only exposed to WR-1065 for a period of fifteen min, at which time the medium was changed and cells were exposed to medium containing the various

**TABLE 3**

A comparison of amifostine's protective effects against cyclophosphamide (cyclo)-induced cytotoxicity and mutagenesis

| Treatment | No. of fibrosarcoma lung colonies | HPRT[1] mutant frequency[2] |
|---|---|---|
| **Untreated** | 68 ± 10 | 15 ± 3 |
| **Cyclo only** (100 mg/kg) | 0.5 ± 0.3 | 160 ± 45 |
| **WR-2721 only** (100 mg/kg) | 61.3 ± 8.9 | 15 ± 2 |
| **WR-2721 administered 30 min before cyclo** | 0.5 ± 0.3 | 35 ± 6 |
| **WR-2721 administered 2 h after cyclo** | 0.9 ± 0.3 | 28 ± 4 |

Means ± SEM

[1] Hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus.

[2] Mutants per $10^7$ viable cells.

Reprinted from Kataoka *et al.* /48/ with permission from W.B. Saunders.

chemotherapeutic agents for prolonged periods of time. Under these conditions the intracellular concentrations of the thiol and disulfide forms of amifostine fall to undetectable levels within 30 min after changing the drug-containing medium. Cytoprotection would not be expected to occur under these conditions regardless of the cells evaluated /6/. Second, the dose of WR-1065 was relatively low and not in the range that is usually used to demonstrate cytoprotection *in vitro*, i.e., 1 mM or greater. Finally, the assay system used in this study is not compelling as a measure of the effectiveness of WR-1065 on cell killing by anti-cancer drugs. It is a cell growth assay that can be affected by alterations in growth rate and/or delay and not necessarily cell killing. Many *in vitro* studies have demonstrated that tumor cells in culture can be significantly protected by WR-1065 /53-55/. As an example, WR-1065 at a dose of 4 mM significantly protected four different human glioma cell lines by protection factors ranging from 1.9 to 2.8, irrespective of whether the tumor cells were p53 wild-type or mutant /55/.

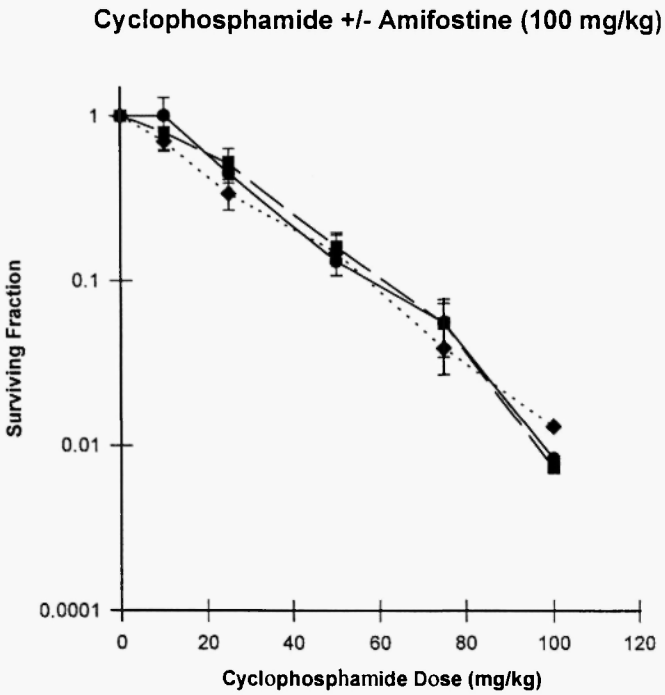


**Fig. 4:** Effect of amifostine on cyclophosphamide-induced killing of murine FSA tumor cells as evidenced by changes in the number of tumor nodules in the lungs of treated mice. Animals were given injections i.v. of 3.5 x $10^5$ viable FSA cells, and four days later were given i.p. graded doses of cyclophosphamide alone (●) or in combination with a 100 mg/kg dose of amifostine i.p. given thirty minutes before (■) or two hours after (◆). Error bars represent one SEM. Reprinted from Grdina *et al.*[50] by permission of W.B. Saunders.

Very little is known as to why some cells are protected by amifostine and others are not, especially when exposures to the active thiol, WR-1065, occur under *in vitro* conditions in which issues related to drug delivery and concentration are not applicable. There is information, however, that suggests that the DNA repair status of a cell may be a determinant. Recombination-deficient repair mutants of *Escherichia coli* (i.e., $REC^-$) bacteria, in contrast to their wild-type parent strains that are protected by factors of 2 or greater, are not protected by the aminothiol cysteamine when they are exposed to

ionizing radiation /56,57/. Likewise, cysteamine was ineffective as a cytoprotector for recombinational repair deficient strains of the yeast *Saccharomyces cerevisiae* /58/. Finally, while diploid wild-type yeast could be protected by cysteamine, haploid wild-type yeast were not protected /58/. These data suggest that an intact DNA repair system is a prerequisite for cytoprotection by aminothiols. This conclusion is further supported by cytoprotection data obtained using repair proficient and deficient (i.e., defective in DNA double strand break repair) mutants of Chinese hamster cell lines designated K1 and xrs-5, respectively /59/. Exposure of cells to 4 mM of WR-1065 for 30 min prior to irradiation resulted in a protection factor of 1.4 for K1 and no protection (i.e., a protection factor of 1) for xrs-5 cells (see Fig. 5) /59/. To further explore the role of DNA repair as a requirement for

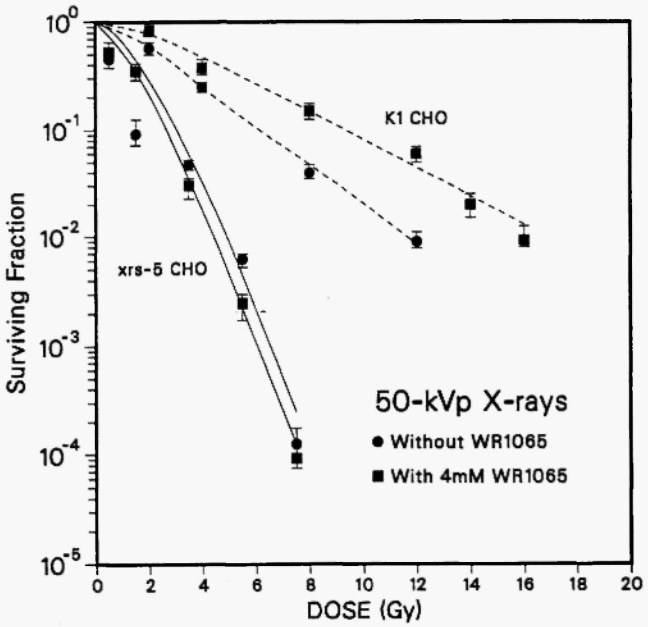


**Fig. 5:** Effect of WR-1065 on X-ray-induced cytotoxicity in double-strand break repair-proficient K1-CHO (Chinese hamster ovary) cells and repair-deficient xrs-5 CHO cells: irradiation only (●); one hour exposure to 4 mM WR-1065 before irradiation (■). All experimental points are the average of three separate experiments, and error bars represent one SEM. Reprinted from Grdina *et al.*[59] by permission of Plenum Press.

cytoprotection by aminothiols, xrs-5 repair deficient cells were exposed to 5-azacytidine to induce wild-type revertants. These were identified and isolated, suggesting that a silent methylated copy of a repair gene may have been reactivated by azacytidine-mediated hypomethylation /60/. These revertants reacquired the ability to be cytoprotected by WR-1065 /60/.

The precise mechanism by which a repair-associated enzyme interacts with WR-1065 to facilitate cytoprotection remains unclear. An attractive hypothesis is that a repair related protein factor would facilitate the sequestering of both thiol and disulfide metabolites of amifostine to the microenvironment of the DNA. The lack of a functional protein factor in repair deficient cells would not negate the ability of the thiol to scavenge free radicals produced by radiation within the cell, but would rather, by diminishing the sequestration of thiols to the target DNA molecules, render any cytoprotection ineffective. The importance of a protein factor in cytoprotection by amifostine is further supported by the demonstration that exposure of cells to the protein synthesis inhibitor cycloheximide leads to a loss of amifostine's cytoprotective efficacy /61/. Thus, even repair proficient cells can be made refractory to the cytoprotective effects of amifostine when their ability to synthesize new proteins is inhibited.

The preponderance of the data to date from both animal models and *in vitro* cell culture systems do not demonstrate that there is any intrinsic difference between normal and malignant cells that can account for differences in cytoprotection by amifostine. However, the repair status of the cells, irrespective of their malignant state, appears to be an important determinant that may account for differences in cytoprotection reported in the literature. As a generalization, it can be concluded that repair proficient tumor cells can be protected by amifostine's active thiol metabolite if it is present at sufficiently high concentrations at the time of administration of a cytotoxic therapeutic treatment /35-40,53-55/. Clinical data obtained to date strongly suggest that this condition is not a problem under the current cytoprotection protocols with amifostine that have been approved by the FDA in the treatment of advanced ovarian and head and neck tumors.

## 4. CHEMOPREVENTION VERSUS CYTOPROTECTION BY AMIFOSTINE

The use of agents to inhibit the carcinogenic process in cells can be defined as chemoprevention /62-66/. The focus of traditional chemopreventive research and subsequent clinical trials is the chronic administration of potential cancer preventive agents at very low doses to reduce the risk of cancer development in individuals at high risk to develop cancer. Under this approach the three general populations targeted for chemoprevention include those at high risk to develop their first cancer due to genetic, environmental, and/or life style factors. However, a fourth population can be identified that is represented by individuals who have had cancer and have been successfully treated using conventional radiation- and/or chemotherapies. As these therapeutic modalities have been made more effective, and more individuals are living for longer periods of time, cancer survivors are faced with a significant new risk of developing therapy-induced secondary cancers /67-70/. Because of the inherent mutagenicity of ionizing radiation and most anti-cancer drugs, concern was voiced as early as 1978 that therapy-induced secondary tumors would become a major health issue /71/. This possibility is best described with regard to children and young adults having potentially curable cancers such as Hodgkin's disease. Teenage women treated for Hodgkin's disease with radiation and chemotherapy exhibited a 75-fold increased risk for the development of breast cancer by age 40 as compared to matched control populations /70/. The issue of therapy-induced secondary cancers could be effectively addressed if novel anti-cancer agents lacking mutagenic and carcinogenic properties were to be identified and developed. However, this approach suffers from the enormous cost and low probability of success in developing a new paradigm in which non-mutagenic and non-carcinogenic agents are equally or more effective than those currently used in cancer therapy. A reasonable alternative is the addition of agents that are known to exhibit anti-mutagenic and anti-carcinogenic properties to standard cancer therapy protocols. Since the target population for this approach is represented by patients having a good prognosis for cure coupled with a relatively long life expectancy, it is essential that the chemopreventive agent used neither diminish nor adversely affect the therapeutic efficacy of the treatment protocols used. It is within this

context that amifostine may be considered a candidate drug for use in chemoprevention.

In contrast to the requirement that amifostine be present at high doses to achieve cytoprotection during cancer treatment, much lower doses can be used for chemoprevention. This is because its chemopreventive/anti-mutagenic properties are relatively uniform over a wide range of concentrations (see Fig. 6) /6/ and are evident even when administered up to 3 hours following exposure to ionizing radiation (see Figs. 7 and 8) /72/. This suggests a separate mechanism of action for chemoprevention as compared to cytoprotection. A large preclinical database exists in the literature that describes the efficacy

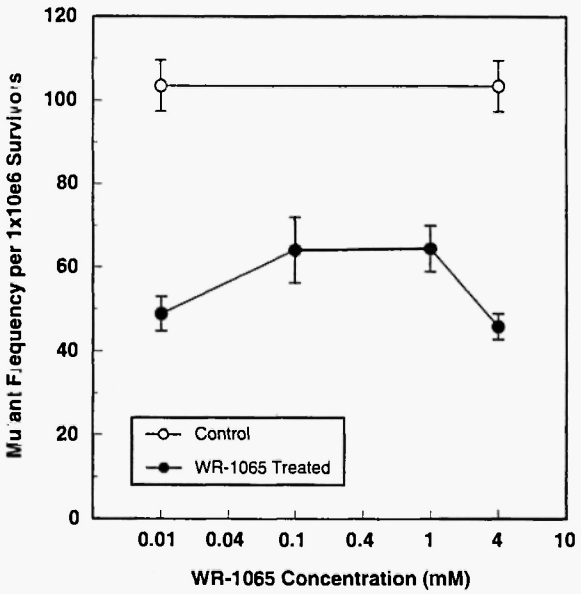


**Fig. 6:** Relationship between WR-1065 concentration and the induction of mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in CHO AA8 cells irradiated with a single 7.5 Gy dose of $^{60}Co$ irradiation. The top line represents the initial frequency per $10^6$ surviving cells of cells not exposed to WR-1065 before irradiation (O), the bottom line represents cells exposed to different doses of WR-1065 (●). All experimental points are the average of three separate experiments, and error bars represent one SEM. Reprinted from Grdina *et al.*[6] by permission of Oxford University Press.

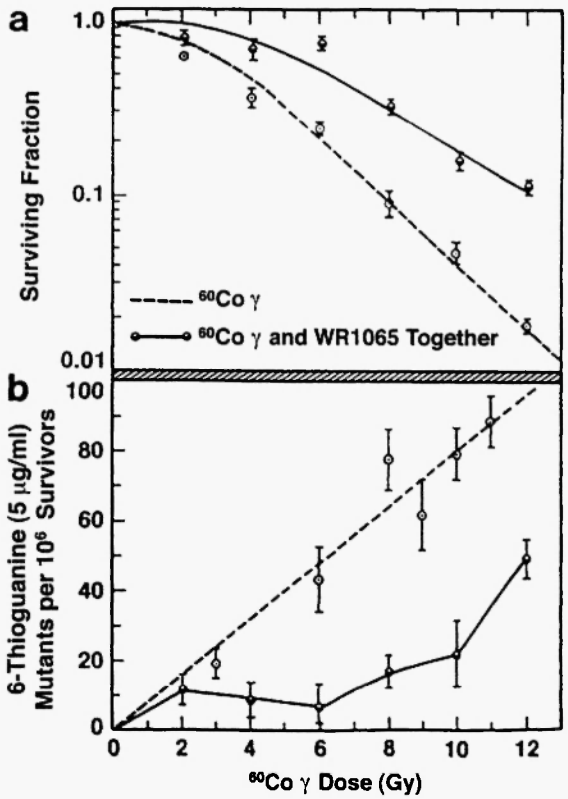


**Fig. 7:** Radiation response of V79 cells exposed to $^{60}$Co γ-rays in the absence or presence of 4 mM WR-1065: **a**. cell survival; **b**. mutation induction at the HPRT locus. WR-1065 was added to cells 30 min prior to and removed 3 hours following irradiation. Background mutation frequency ranged from 3 to 25 mutants per $10^6$ cells, and all mutation frequencies were corrected for background. Error bars represent one SEM. Reprinted from Grdina *et al* [72] by permission of Oxford University Press.

of amifostine for use as an anti-mutagenic /6,7,49,50,73-76/, anti-transforming /77,78/, and anti-carcinogenic agent /8,9,79/. Amifostine appears to affect the first step of the carcinogenic process, known as initiation. Initiation events occur immediately following exposure of cells to genotoxic agents as a result of either unrepaired or incorrectly repaired DNA damage. If such damage is carried forward following

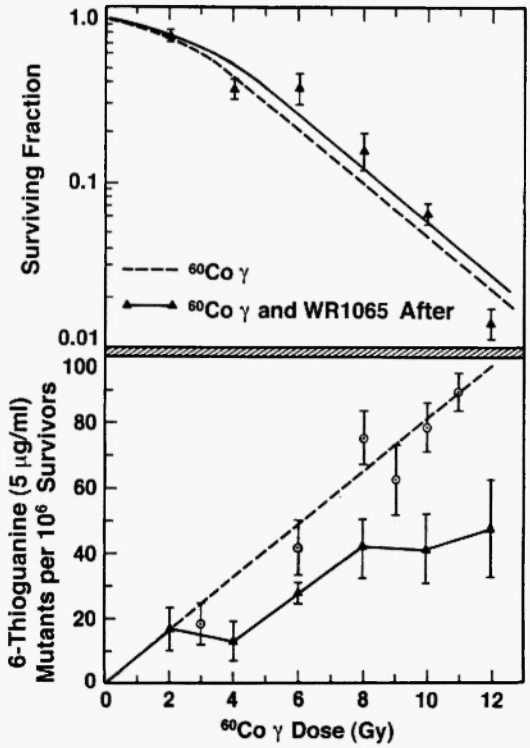


**Fig. 8:** Radiation response of V79 cells exposed immediately following $^{60}$Co irradiation to 4 mM WR-1065 for 3 h: **a**. cell survival; **b**. mutation induction. The dashed line represents the radiation-only survival curve and is presented for comparison. Reprinted from Grdina *et al* [72] by permission of Oxford University Press.

cell division, it will become fixed and represent an increased probability for cellular transformation to occur. In general, chemopreventive agents that are only effective in preventing initiation have limited utility. However, the treatment of cancer utilizes highly mutagenic/carcinogenic agents, i.e., ionizing radiation and DNA damaging drugs, that are administered at known times and doses, thus allowing for the efficacious use of inhibitory agents of initiation such as amifostine. The ability of amifostine to prevent mutations does not appear to be related to its ability to simply scavenge free radicals, and in that way reduce initial damage, but rather appears to be involved in

the repair of that damage. In particular, amifostine appears to primarily affect the fidelity rather than the magnitude of DNA repair processes. This is demonstrated in Figure 8 /72/; cell survival is not affected but the frequency of mutation induction is reduced. Presumably, if the magnitude of DNA repair were increased, it would also be accompanied by an increase in cell survival. An increase in the fidelity of repair would be manifested by a lower mutation rate. Indirect evidence also indicates that free radical scavenging by amifostine is not the underlying mechanism of action that accounts for this effect, since the antimutagenic effect is also demonstrable under conditions of acute hypoxia in which free radical production is not a factor /80/.

The potential applications of amifostine as a chemopreventive agent for use in cancer treatment are supported by a number of animal studies. Amifostine has been demonstrated to be effective in preventing the induction of pre-neoplastic lesions by ionizing radiation in the livers of neonatal rats /81/. Injection of one day-old rat pups with 100 mg/kg of amifostine 30 min prior to receiving a single dose of either 150 or 300 cGy of ionizing radiation resulted in a 9.7-fold reduction in the number of preneoplastic foci induced. These foci represented phenotypic changes in the histochemical markers γ-glutamyltranspeptidase and iron exclusion. Milas *et al.* /8/ demonstrated that amifostine at a dose of 400 mg/kg could reduce by 3-fold the frequency of secondary tumor formation in irradiated fields in mice (see Fig. 9). Comprehensive carcinogenesis studies were carried out in mice, 200 age and sex matched animals per group, at the Argonne National Laboratory. Animals were exposed to either 2 Gy of low LET gamma rays or 0.1 Gy of fission spectrum neutron irradiation in the presence or absence of treatment with 400 mg/kg doses of amifostine. Animals injected with amifostine 30 min prior to exposure to whole body irradiation were protected against radiation-induced carcinogenesis and subsequent life shortening /9,82,83/. A total of 164 tumor codes were used to assess the cause of death. Female C57BL x BALB/c $F_1$ mice which were injected with amifostine at a dose of 400 mg/kg were afforded significant protection (P=0.0016) against the development of all radiation-induced malignancies compared with untreated controls (see Table 4) /9/. With respect to protection against a specific class of tumors, lymphoreticular neoplastic disease was the most preventable (P=0.0165) (Table 5) /9/. Lymphoreticular tumors are highly prevalent

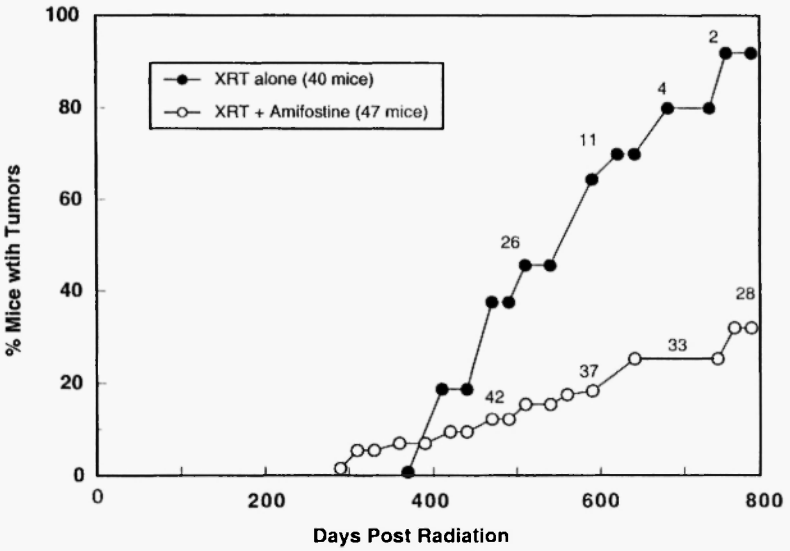


**Fig. 9:** Cumulative incidence of radiation-induced tumors (fibrosarcoma) as a function of time following irradiation (34 Gy to 57 Gy single dose γ-rays) of the hind leg of C3Hf/Kam mice with or without pretreatment with amifostine (400 mg/kg i.p.). Reprinted from Milas *et al.*[8] by permission of the American Association for Cancer Research.

in this mouse system, and include fibrosarcoma-lymph node, histiocytic leukemia, histiocytic lymphoma, lymphocytic-lymphoblastic lymphoma, myelogenous leukemia, plasma cell tumors, undifferentiated leukemia, undifferentiated lymphoma, unclassified lymphoma, mixed histiocytic-lymphocytic leukemia, and mixed histiocytic-lymphocytic lymphoma. The overall life span of amifostine-treated animals was extended by 65 days relative to their matched controls following irradiation. Extrapolated to a human time frame, this increase would represent a life extension of approximately 6 years /9/. A single injection of amifostine at a dose of 400 mg/kg had no effect, however, on either the cumulative survival or tumor incidence in unirradiated animals.

An investigation of the anti-carcinogenic properties of amifostine has been extended most recently to include a demonstration of the prevention of radiation-induced mammary tumor formation in rats during pregnancy /79/. Pregnant rats were injected i.p. with 50 mg of

**TABLE 4**

The protective effects of amifostine against γ-radiation-induced carcinogenesis in female mice

| Days | γ-Irradiated [a] | | | Neutron Irradiated [b] | | |
|---|---|---|---|---|---|---|
| | Control | 206 cGy | 206 cGy + WR-2721 | Control | 10 cGy | 10 cGy + WR-2721 |
| 700 | 19.2 | 34.0 | 25.5 | 16.9 | 25.0 | 14.3 |
| 900 | 55.1 | 71.0 | 61.3 | 50.3 | 60.3 | 52.9 |
| 1100 | 91.1 | 99.4 | 94.5 | 84.8 | 92.3 | 89.3 |

Percent tumor-related deaths at days postexposure

[a] Animals at risk: Control, 182; 206 cGy 186; 206 cGy + WR-2721, 180.

[b] Animals at risk: Control, 182; 10 cGy, 184; 10 cGy + WR-2721, 186.

**TABLE 5**

The protective effects of amifostine against γ-radiation-induced lymphoreticular tumors in female mice

| Days | Control | 206 cGy | 206 cGy + WR-2721 |
|---|---|---|---|
| **700** | 16.6 | 22.8 | 13.0 |
| **900** | 50.9 | 53.6 | 44.9 |
| **1100** | 84.9 | 96.7 | 83.7 |
| **Total deaths due to lymphoreticular tumors** | 84 | 88 | 91 |

Animals at risk: Control, 182; 206 cGy, 186; 206 cGy + WR-2721, 180.

amifostine 30 min prior to whole body irradiation with 1.5 Gy of γ-rays. All rats were also implanted with pellets of the tumor promoter diethylstilbestrol one month after the termination of nursing and were observed for an additional year for the development of palpable tumors. No tumors developed in unirradiated rats. However, rats exposed to 1.5 Gy had a 71.4% incidence of mammary tumors within one year of follow-up. Pretreatment with amifostine significantly delayed the onset and reduced the incidence of mammary tumor development in these animals by 23.8% ($P<0.005$).

In all of these animal studies amifostine was only administered as a single dose just prior to irradiation, indicating that the effect measured was only on the initiation phase of the carcinogenic process. Two distinct and independent mechanisms of action of amifostine presumably account for this effect. First, the presence of amifostine at the time of irradiation would protect against radiation-induced free radical damage to DNA and thus reduce the magnitude of the carcinogenic insult suffered by the exposed cells at risk /24/. Second, the ability of amifostine's thiol and disulfide forms to stabilize chromatin structure and enhance the fidelity of DNA repair processes would reduce the probability of unrepaired or misrepaired DNA lesions remaining following cellular division that could lead to genomic instability and mutagenic development /6,7,13,61/. The ability of amifostine to inhibit the initiation process of carcinogenesis makes it an important

candidate for use in the chemoprevention of radiation- and chemotherapy-induced secondary neoplasias in cancer patients characterized as having a good prognosis and a relatively long life expectancy. While no data exist that suggest that amifostine might be beneficial in inhibiting the promotion and/or progression stages of carcinogenesis, the requirement that it be administered via injection would significantly reduce its utility for use as a chronically administered chemopreventive agent. Regardless, within the setting of a conventional cancer treatment regimen, amifostine would be readily amenable for use not only as a cytoprotector, but also as a chemopreventive adjuvant for the prevention of therapy-induced secondary tumors.

## 5. MOLECULAR PROCESSES AFFECTED BY AMIFOSTINE'S THIOL AND DISULFIDE FORMS AND THEIR POSSIBLE ROLES IN CYTOPROTECTION AND CHEMOPREVENTION

As described earlier, amifostine is a prodrug that requires activation by dephosphorylation through the action of alkaline phosphatase. The resultant free thiol and disulfide forms can then participate in a number of "normal" intracellular processes, in addition to those initiated by radiation- and/or drug-induced damage-related responses. Anti-oxidant thiols such as WR-1065 can affect intracellular reduction-oxidation (redox) states that in turn can alter gene expression /84,85/, synthesis of other intracellular thiols /86,87/ and enzyme activities (i.e. redox control of sensitive cysteine residues) /88,89/. Redox-based regulation of signal transduction and gene expression is recognized as an important regulatory mechanism in cell biology /84,88-90/. Redox-sensitive targets will respond differently as the intracellular redox environment of the cell is altered by its exposure to varying levels of exogenously added thiols. As an example, it has been reported that the thiols *N*-acetylcysteine (NAC) and oltipraz can activate the redox sensitive transcription factor NFκB and enhance manganese superoxide dismutase (MnSOD) gene expression /91,92/. Using a standard gel shift assay as described elsewhere /91,92/ for these thiols, NFκB activation can be demonstrated following a 30 min exposure of human microvascular endothelial cells (HMEC) to WR-1065, both the L- and D- sterioisomeric forms of NAC, and captopril (Fig. 10). Exposure of cells to WR-1065 can also lead to the accumulation and activation of the p53 protein that also acts as a transcription

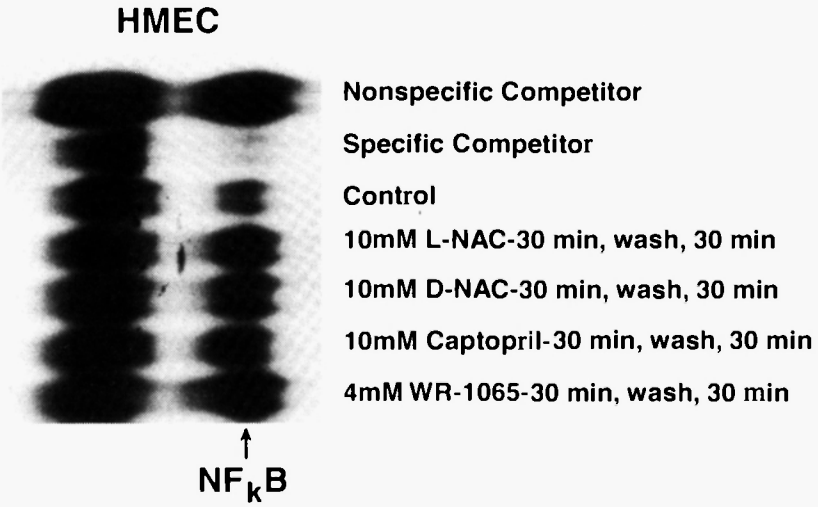


**Fig. 10:** Effect of *N*-acetyl-L-cysteine (L-NAC), *N*-acetyl-D-cysteine (D-NAC), captopril, and WR-1065 on activation of the redox sensitive transcription factor NFκB in human microvascular endothelial cells (HMEC) 30 min following a 30 min thiol exposure as described using a standard gel shift assay.

factor /93/. This activation has been reported in MCF-7 breast cancer cells and 3T3 mouse fibroblast cells and is accompanied by induced expression of the cyclin-dependent kinase inhibitor $p21^{waf-1}$ /93/. It is therefore not surprising that exposure of cells to amifostine's free thiol WR-1065 can lead to changes in gene expression, as demonstrated in Figure 11. Using the technique of differential display to measure changes in mRNA levels, it is possible to identify and demonstrate changes in the expression of a number of genes following 30 min exposure of cells to different concentrations of either the thiol or disulfide forms of amifostine /85,94/. Thymidine kinase (tk) gene expression is maximally enhanced 4 h following exposure of CHO cells to a 40 μM dose of WR-1065 by a factor of 3.5, while a 4 mM dose leads to only a 2.4-fold enhancement /85/. Both the thiol and disulfide forms of amifostine can repress c-myc gene expression. The disulfide form is the more effective, as evidenced by its ability to repress c-myc gene expression to 27% of control level as compared to only 46% by the thiol /94/. Consistent with a redox-driven process

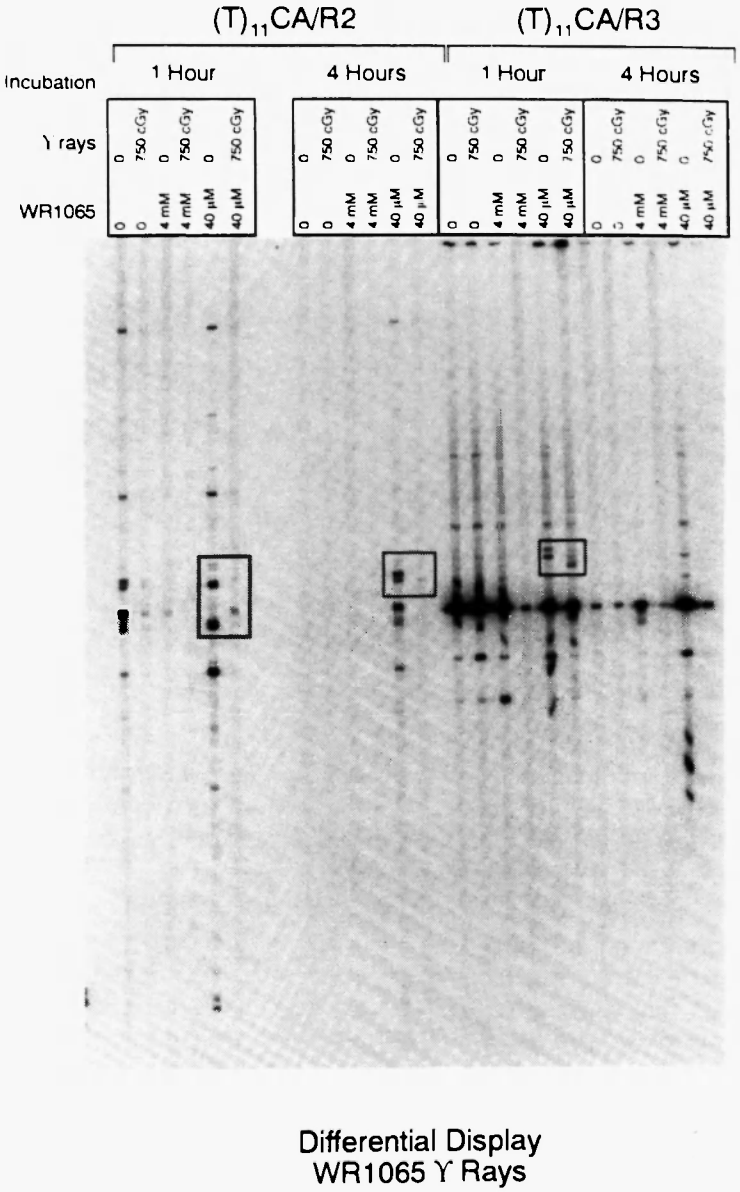


**Fig. 11:** Differential display polymerase chain reaction of RNA from cells exposed to ionizing radiation and/or WR-1065 as shown in the figure. The 180-bp size marker is indicated on the gel.

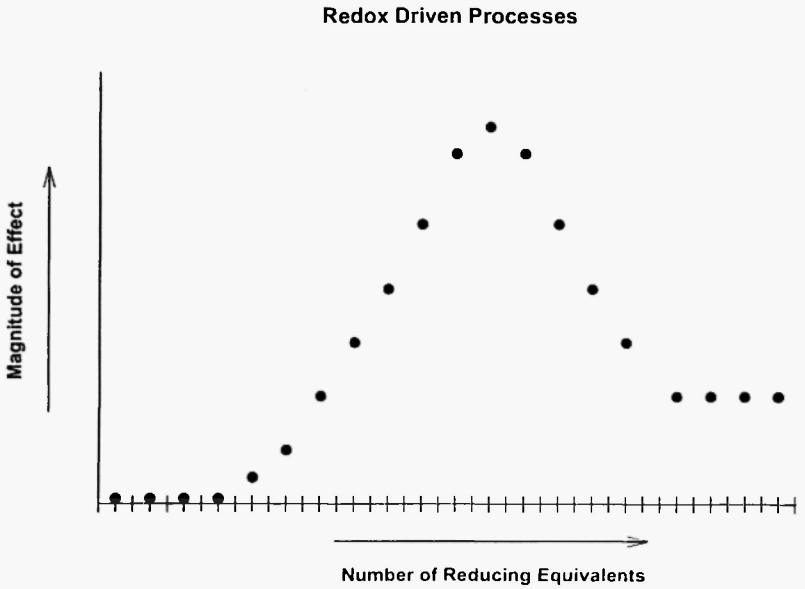


**Fig. 12:** Relationship between the number of reducing equivalents added and magnitude of effect in model redox driven processes.

(see Fig. 12), 40 μM was more effective than 4 mM in altering gene expression, even though the timing of the effect was gene specific, i.e., 1 h for c-myc /85/ and 4 h for tk /94/.

Thiol-mediated effects are not limited to free radical scavenging and alterations to the redox environment of cells. Thiols are also capable of chelating metals such as boron, calcium, chromium, cobalt, lead, magnesium, and zinc. Thiols capable of chelating metals currently in clinical use include NAC /95-97/, captopril /98,99/, cystamine /100/ and amifostine /101,102/. An important implication of this is that these thiols can facilitate the removal of metal cofactors from metal-requiring enzymes, and thereby effect changes in their subsequent enzymatic activities. The zinc-requiring matrix metalloproteinases (MMPs) are an important example of this class of enzyme. MMPs can degrade the extracellular matrix and facilitate invasion of tumor cells in the metastatic process /103/. An example of the abilities of these thiols to inhibit the enzymatic activities of MMP-2 and -9 secreted by cultured human glioma cells are demonstrated in the

zymogram gel presented in Figure 13. Following 24 h exposure of these cells to either WR-1065 (SH; 4 mM), *N*-acetylcysteine L form (L-NAC; 10 mM), *N*-acetylcysteine D form (D-NAC; 10 mM), captopril (10 mM), or EDTA (50 mM), the activities of MMP-2 and -9 were significantly inhibited. In the case of captopril, and presumably the other thiols as well, this inhibition can be reversed by the addition of $ZnCl_2$ /99/.

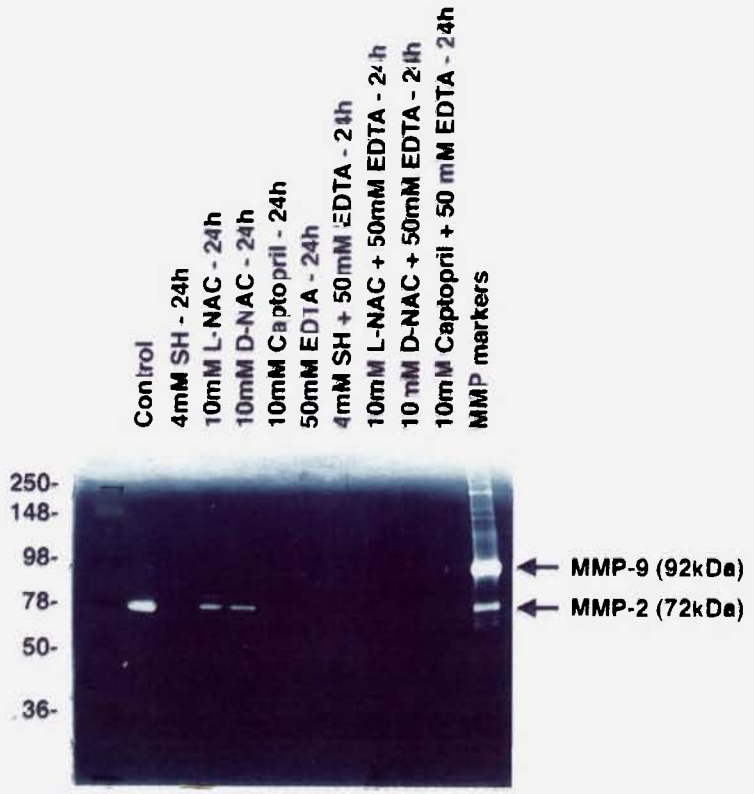


**Fig. 13:** Effect of WR-1065 (SH), L-NAC, D-NAC, Captopril, and the metal chelator EDTA on inhibiting matrix metalloproteinases (MMPs) 2 and 9 activities as measured by gelatin zymography. Human glioma cells were exposed to each of these agents for 24 h. MMP 2 and 9 activities were measured in serum-free medium isolated from confluent cultures. The relative magnitude of enzymatic activity is readily observable by the extent of the white area as depicted on the blue background. WR-1065 (SH), captopril, and EDTA completely inhibited MMP 2 and 9 activities.

While the free thiol form of amifostine can affect a myriad of intra- and extracellular processes through its ability to scavenge damaging free radicals, alter redox processes, and chelate metal cofactors required for enzyme activity, the disulfide form has also been implicated in affecting a number of cellular processes by virtue of its polyamine-like structure and properties. The disulfide form of amifostine is structurally similar to the polyamine spermine (see Fig. 1). Polyamines are ubiquitous polycationic molecules that participate in a number of cellular processes including effects on chromatin stabilization, DNA synthesis, kinase activity, gene expression, and protein conformation /104/. The close structural similarity appears to be correlated with many functional similarities. For example, amifostine has been demonstrated to be an effective inhibitor of putrescine uptake into the lung tissue of rats /105/, and to be a substrate for polyamine oxidase /106/. In contrast to its free thiol form, WR-1065, which is taken up into cells predominantly by passive diffusion, the disulfide form is transported actively into cells via a polyamine transport system (see Fig. 14) /107/. It is transported intracellularly at the same velocity as spermidine, and is capable of competing with and inhibiting the cellular incorporation of spermidine. Inactivation of the polyamine transporter system by the use of inhibitory agents or site-directed mutagenesis leads to a comparable inhibition of the uptake of both the disulfide and spermidine /107/. Similarities also exist between the thiol and disulfide forms and corresponding polyamines having the same net charge with regard to the propensity of electrostatic binding to the minor groove of DNA /108-110/. The disulfide form occupies a binding site of approximately 10 nucleotides, while the free thiol has one of only 5 nucleotides /110/. Each competes equally with polyamines of comparable electrostatic charge for the same DNA binding sites /111/. Measurements of the intracellular distribution of the disulfide demonstrate that it is concentrated over 10-fold within the nucleus as compared to the cytoplasm.

The interconversion between the thiol and disulfide forms of amifostine is due to intracellular redox reactions. Cells exposed only to WR-1065 at cytoprotective concentrations will exhibit an intracellular ratio of ten thiol molecules to one disulfide molecule if the measurements are based on total cellular volume /6/. Thus, a measured 1 mM intracellular concentration of thiol is accompanied by only a 0.1

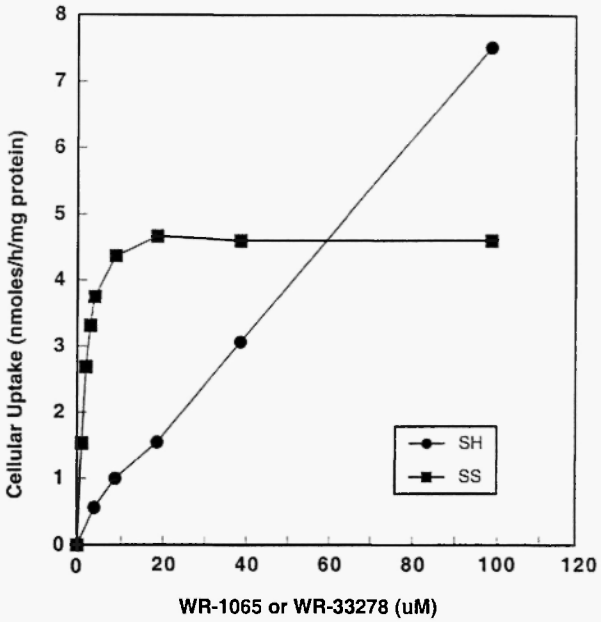


**Fig. 14:** Comparison of the uptake of low levels of WR-1065 (SH) and its disulfide WR-33278 into rat hepatoma (HTC) cells. Data are presented as the velocity (nmol/h/mg protein) of either WR-1065 (●) or WR-33278 (■) uptake. Reprinted from Mitchell *et al.*[107] by permission of Oxford University Press.

mM concentration of disulfide. However, if the measurements are made only within the nucleus, the values obtained are 2.2 mM for the thiol and 2.6 mM for the disulfide /111/. This is interpreted as being a consequence of the active sequestration of the thiol and disulfide metabolites of amifostine within the nuclear volume of cells. A consequence of this ability to both concentrate within the microenvironment of the chromatin and electrostatically bind to DNA is a resultant distortion of replicon supercoiling. This in turn can lead to conformational changes in nucleoid structure. This has been observed and has been reported to occur with no detectable change in nucleoid content /112/. Such conformational changes in chromatin structure can play a role regarding changes in subsequent gene expression and DNA repair.

Amifostine's thiol and disulfide metabolites can also affect enzymes involved in DNA repair and synthesis. DNA-specific exonuclease activity, for example, is inhibited following exposure of cells to cytoprotective doses of WR-1065 /113/. Other effects on DNA repair are most likely due to a combination of amifostine's metabolites binding directly to damaged sites in irradiated DNA as well as to sites on nuclear proteins /114/. Like the polyamine spermine, amifostine can affect DNA synthesis and subsequent cell cycle progression by inhibiting the enzymatic activity of the enzyme topoisomerase II-α /115,116/. Specifically, 30 min exposure of cells to doses as low as 4 μM of WR-1065 leads to 50% inhibition of topoisomerase II-α activity, as determined using a kinetoplast DNA decatenation assay /117/. The decrease in enzyme activity was correlated with 45% decrease in the phosphorylation level of topoisomerase II-α, determined using an immunoprecipitation assay and an antibody specific to its 170 kDa band (see Fig. 15) /117/. The catalytic inhibitory effects of amifostine on topoisomerase II-α activity have been verified in a

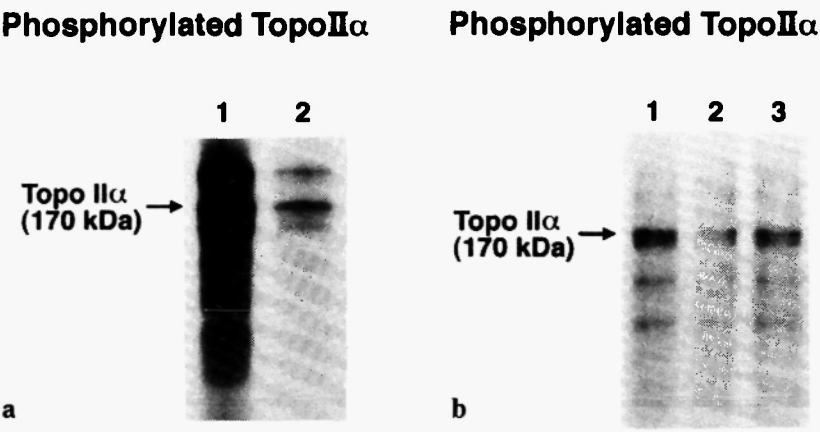


**Fig. 15:** Autoradiograph of representative gels describing phosphorylated topoisomerase (topo)II-α extracted from CHO AA8 cells. The samples were adjusted for equal counts per minute prior to immunoprecipitation with topoIIα (170 kDa) specific antibody and separation by SDS-polyacrylamide gel electrophoresis. **a**. Lane 1, untreated control cells; lane 2, cells exposed to 4 mM WR-1065 for 30 min. **b**. Lane 1, untreated control cells; lane 2, cells treated with 40 μM WR-1065 for 30 min; lane 3, cells treated with 0.4 μM WR-1065 for 30 min. Reprinted from Murley *et al.*[117] by permission of Blackwell Science Ltd.

number of mammalian cell systems using a cell-based micronucleus assay /118/. These data, along with the observations that amifostine can lead to activation of $p21^{waf-1}$ /93/ and repression of c-myc gene expression /94/, are consistent with the observations that exposure of cells to amifostine can lead to a transitory inhibition of cell growth and cell-cycle progression. This in turn could afford additional time for DNA repair to occur before DNA damage would be fixed at mitosis, thus facilitating an enhanced fidelity of repair and lowering the probability of the formation of pre-carcinogenic mutations.

As described above, the thiol and disulfide metabolites of amifostine, by virtue of their redox and polyamine-like properties, are capable of participating in a range of intra- and extracellular processes. Amifostine has been implicated in affecting signal transduction processes, gene expression, genomic stability, enzymatic activities, DNA synthesis and repair, and cell-cycle progression. Its affects on these processes, along with its ability to directly prevent DNA damage through the physiochemical processes of radical scavenging, chemical repair by hydrogen atom donation, and induced hypoxia by auto-oxidation, make amifostine a unique drug for use in both cyto-protection and chemoprevention. However, because of the broad scope of amifostine's ability to affect these many processes, care must be taken to identify those effects that will have relevance to the *in vivo* condition. Serum concentrations in excess of 100 μM and exposure times of over 3 h are not readily achievable in humans or animals due to inherent dose limiting toxicities and drug delivery restrictions. Therefore, effects observed following *in vitro* exposure times of 24 h or more using extracellular concentrations in the millimolar range must be interpreted with caution, since they will be very difficult to extrapolate to *in vivo* conditions.

## 6. AMIFOSTINE MODEL OF CYTOPROTECTION AND CHEMOPREVENTION

A generalized model of cytoprotection and chemoprevention by amifostine is presented in Figure 16. Amifostine (WR-2721) is a prodrug that requires activation by membrane-bound alkaline phosphatase. Following dephosphorylation the free thiol form (SH) enters the cell by way of passive diffusion /24/. In contrast, the disulfide (SS)

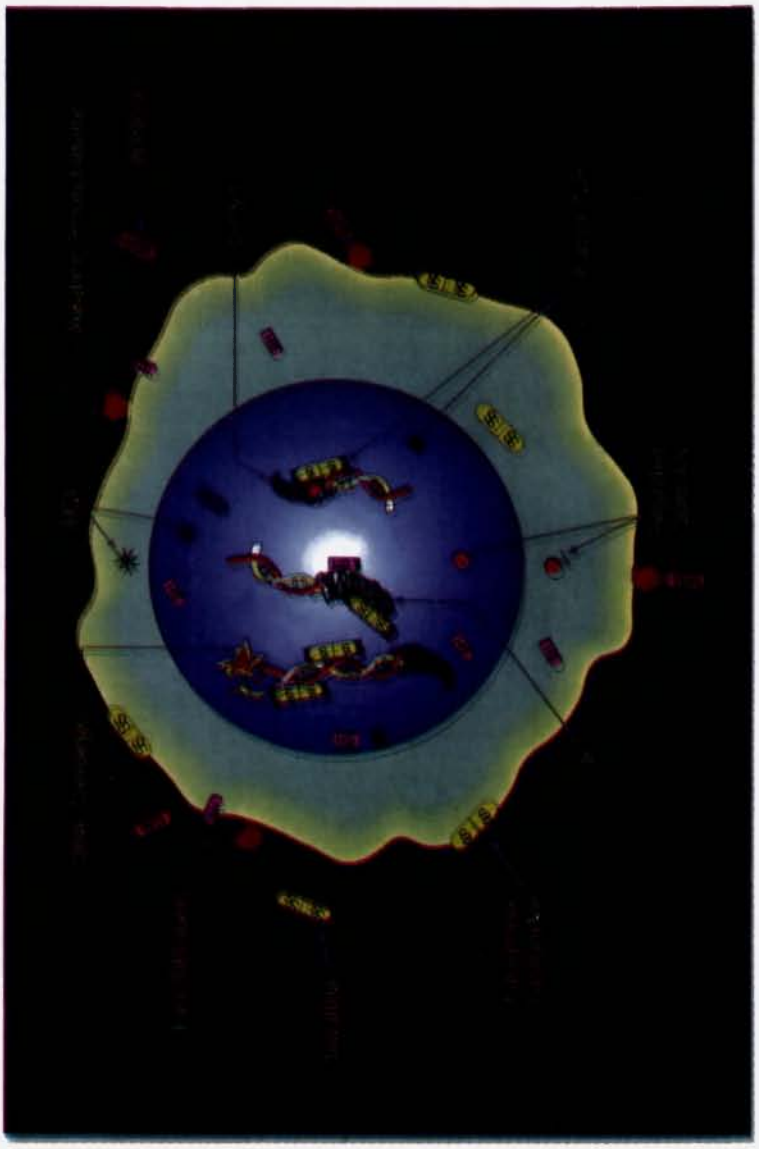

**Fig. 16:** Uptake and sequestration of thiol (SH) and disulfide (SS) metabolites of amifostine. ROI = reactive oxygen intermediates; tk = thymidine kinase gene; GR = good repair factor. Model provided by DJ Grdina.

form of the drug is actively taken into the cell via its polyamine transport system /107/. Once inside the cell, SH, SS, and mixed disulfide forms will participate in redox-driven reactions affecting a myriad of cellular processes, including signal transduction, gene expression, and enzyme activity /85,88,90,93,95,115,117/. In contrast to negatively or neutrally charged molecules, the net 2+ charged SH and 4+ charged SS molecules can localize within the negatively charged microenvironment of the mitochondrial and nuclear DNA /110,111/. However, the ability of these molecules to sequester within the microenvironment of nuclear DNA appears to be dependent upon the DNA repair proficiency of the cell /56-59/. It is hypothesized that a repair related protein or class of proteins, designated GR for "good repair", is required to facilitate this process. If amifostine is present during the administration of radiation or chemotherapy, the SH form will facilitate cytoprotection through the scavenging of reactive oxygen intermediates (ROI) and/or through the conversion of reactive species into stable molecules. The underlying mechanism of cyto-protection is, therefore, the prevention of or reduction in the amount of damage sustained by cells that leads to their death.

The underlying mechanism of action of chemoprevention, in contrast, involves the efficient processing of damage in non-lethally damaged cells to insure the fidelity of repair. The electrostatic binding to and stabilization of DNA by the SS form /108,110,111/ can help in facilitating a more efficient repair of damaged sites. Alterations in enzymatic activities involved in repair /113,114/ and DNA synthesis, such as amifostine's effects on topoisomerase II-α activity /115,117, 118/, will lead to a transitory inhibition of cell cycle progression, thus allowing for more time in which to correctly repair damage that would become fixed at mitosis. Changes in gene expression, especially those genes involved in the control of cell growth, such as the repression of c-myc, could also play a role in allowing for more time to facilitate an enhancement in the fidelity of the DNA repair process. The con-sequences, therefore, of these events would be the stabilization of the genome as evidenced by a reduced mutation frequency and ultimately a reduced incidence of cellular transformation and carcinogenic development.

Amifostine, by virtue of its polyamine-like structure and associated free sulfhydryl group, is a multifaceted drug with wide ranging poten-tial. While originally designed as a radioprotective drug to prevent free

radical damage to the DNA of cells during irradiation, it has now been demonstrated to affect a myriad of processes in studies ranging from apoptosis to zymography. The challenge is not to be distracted by its vast potential, but rather to focus on maximizing its effective use in the protection of the cancer patient.

**ACKNOWLEDGEMENTS**

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