Arch Pharm Res Vol 22, No 6, 533-541, 1999 Review

Metabolism-Based Anticancer Drug Design

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(Received June 18, 1999)

Many conventional anticancer drugs display relatively poor selectivity for neoplastic cells, in particular for solid tumors. Furthermore, expression or development of drug resistance, increased glutathione transferases as well as enhanced DNA repair decrease the efficacy of these drugs. Research efforts continue to overcome these problems by understanding these mechanisms and by developing more effective anticancer drugs. Cyclophosphamide is one of the most widely used alkylating anticancer agents. Because of its unique activation mechanism, numerous bioreversible prodrugs of phosphoramide mustard, the active species of cyclophosphamide, have been investigated in an attempt to improve the therapeutic index. Solid tumors are particularly resistant to radiation and chemotherapy. There has been considerable interest in designing drugs selective for hypoxic environments prevalent in solid tumors. Much of the work had been centered on nitroheterocyclics that utilize nitroreductase enzyme systems for their activation. In this article, recent developments of anticancer prodrug design are described with a particular emphasis on exploitation of selective metabolic processes for their activation.

Key words : Prodrugs, Cyclophosphamide, Bioreduction, Sulfoxide, Tirapazamine

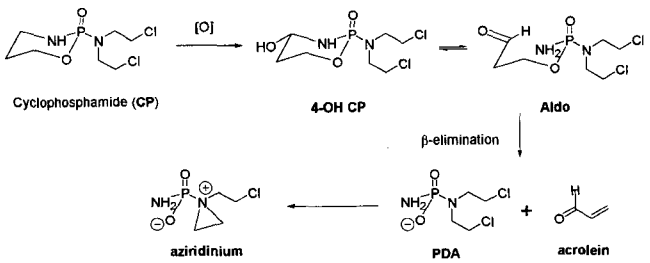
INTRODUCTION

Despite the advances made in cancer chemotherapy in recent decades, many anticancer drugs display relatively poor selectivity for neoplastic cells. Such poor selectivity stems from the fact that anticancer drugs are primarily cytotoxic agents interfering with some aspects of cell division process that also takes place in normal cells. Cancer chemotherapy is therefore usually associated with severe side effects. Prodrug approach has been applied to alleviate toxicity of the anticancer drugs and to improve their target specificity. Prodrugs are non-cytotoxic themselves, but can be converted enzymatically or non-enzymatically to cytotoxic species *in vivo*. Numerous prodrug strategies have been carried out on the basis of elevated enzyme levels in tumor cells and hypoxic environments of solid tumors.

Biooxidative/Hydrolytic Prodrugs

Cyclophosphamide (CP) belongs to a class of oxazaphosphorines and is one of the most widely used alkylating anticancer agents. Cyclophosphamide was designed originally as a prodrug of nitrogen mustard on the basis of findings that a number of tumors displayed high levels of phosphoramidase activity (Gomori, 1948). Although cyclophosphamide showed excellent anticancer activity, its activation is not through phosphoramidase-mediated hydrolysis as originally proposed, but rather is initiated by hepatic microsomal mixed-function oxidase (MFO) catalyzed C_4-hydroxylation. The resulting 4-hydroxycyclophosphamide (4-OH CP) undergoes ring opening to aldophosphamide (Aldo), followed by generation of cytotoxic phosphoramide mustard (PDA) and acrolein by β-elimination (Scheme 1).

The cytotoxic activity of CP is attributed to the aziridinium ion species derived from PDA, the ultimate



Scheme 1. Mechamism of anticancer activity for cyclophosphamide

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alkylating species that cross-links interstrand DNA (Vu *et al.*, 1981; Engle *et al.*, 1982; Hemminki, 1985). Acrolein, a by-product of β-elimination, does not play a significant role in the anticancer activity of CP (Wrabetz *et al.*, 1980), although highly cytotoxic to cultured tumor cells (Conners *et al.*, 1974; Sladek *et al.*, 1985). However, acrolein is responsible for hemorrhagic cystitis, a side effect which is often dose-limiting in cyclophosphamide therapy (Cox, 1979).

The high oncotoxic specificity of cyclophosphamide (CP) is as yet not clear and remains to be elucidated. The identity of the circulating metabolite(s) that enter cells and ultimately exert cytotoxic activity remains controversial. Considerable evidence has been presented supporting the hypothesis that 4-OH CP/Aldo species is the transport form of CP, and that generation of PDA occurs within cells sensitive to CP (Brock, 1976; Brock and Horhorst, 1977; Domeyer and Sladek, 1980; Draeger *et al.*, 1976; Jardine *et al.*, 1978; Low *et al.*, 1982; Powers and Sladek, 1983; Sladek, 1986 & 1988; Sladek *et al.*, 1989). Other data, however, suggest that the contribution of extracellular PDA may also be important, especially when the higher AUC value for PDA is taken into account (Friedman *et al.*, 1976; Hipkens *et al.*, 1981; Struck *et al.*, 1975 and 1983; Alberts *et al.*, 1984).

One of the primary deactivation pathways of CP is the oxidation of 4-OH CP/Aldo to carboxyphosphamide by aldehyde dehydrogenase (AlDH). Recent data suggest that induction of AlDH synthesis may be responsible for the development of resistance to 4-OH CP/Aldo (but, not to phosphoramide mustard) observed in certain L1210 cell lines (Hilton, 1984; Sladek and Landkamer, 1985; Colvin and Hilton, 1988). Despite the assumption that the extracellularly generated PDA will not be readily accessible to the intracellular environment due to its anionic character at physiological pH (pK_a=4.75), it has been shown that extracellularly delivered phosphoramide mustard derivatives are effective against many experimental tumors (Friedman et al., 1976). However, PDA is chemically unstable and possesses relatively short half-life ($t_{1/2}$=17.5 min in 100 mM HEPES buffer at 37°C), limiting its effectiveness as a potential chemotherapeutic agent.

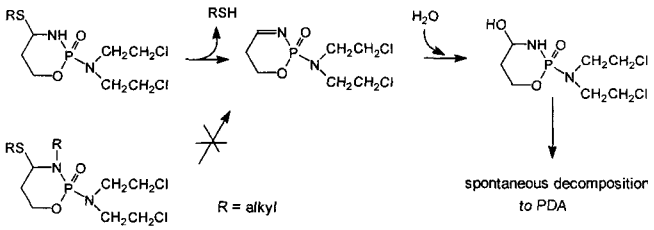
Properly designed bioreversible prodrugs of phosphoramide mustard (PDA) may therefore serve as useful antitumor drugs with improved therapeutic index, especially against cyclophosphamide resistant cell lines. Several phosphoramide mustards, i.e., benzyl-2,4-difluorobenzyl-, and methyl phosphoramide mustards were examined as lipophilic, chemically stable prodrugs of phosphoramide mustard (PDA) (Kwon *et al.*, 1991). These phosphorodiamidic esters were designed to undergo bioactivation by hepatic microsomal enzymes to produce PDA. The rate of formation of alkylating species, viz., PDA, from these prodrugs and their in vitro cytotoxicity toward Balb/3T3 cells were comparable to or better than that of cyclophosphamide. Preliminary antitumor screening against L1210 leukemia in mice, however, showed that these prodrugs were devoid of significant antitumor activity *in vivo*. The lack of any significant *in vivo* antitumor activity of these prodrugs suggests that *in vivo* activation/pharmacokinetics of these prodrugs are not as efficient as those of cyclophosphamide.

Aldophosphamide acetal diacetate and a number of structural analogs were prepared as prodrugs of the corresponding aldehydes, which were designed to be bioactivated by the ubiquitous carboxylate esterases (Wang and Farquhar, 1991). Half-lives of hydrolysis to form the aldehyde in phosphate buffer at 37°C, pH 7.4 ranged 29-42 h, while ranged from 35-42 s with pig liver carboxylate esterase. Thus, the compounds were sufficiently stable under model physiological conditions, however, were readily hydrolyzed with esterases. Although closely structurally related, these analogs exhibited a wide range of cytotoxicity to L1210 leukemia cells *in vitro*.

Overproduction of aldehyde dehydrogenase is a common mechanism of oxazaphosphorine resistant development (Sladek, 1993). Several perhydrooxazine analogs of aldophosphamide were synthesized as alternative prodrugs of phosphoramide mustards, especially against cyclophospamide-resistant tumor cell lines (Borch and Valente, 1991). The activation was designed to be initiated by acid-catalyzed opening of the perhydrooxazine ring to form an enamine that should spontaneously generate cytotoxic phosphoramidates. These compounds were more cytotoxic than 4-hydroperoxycyclophosphamide, the pre-activated cyclophosphamide, against both wild type and cyclophosphamide-resistant L1210 and P388 cells. The *in vivo* anticancer activity of these compounds against L1210 cells, however, was lower than that of 4-hydroperoxycyclophospamide.

A number of sulfonyl-containing phosphoramidates were synthesized as yet another series of alternative prodrugs of phosphoramide mustards (Kwon, 1999a). These compounds were stable in solid state, however, underwent base-catalyzed activation at varying rates (half-lives: 30 min to 30 h) under model physiological conditions. Some of the compounds were more cytotoxic than 4-hydroxycyclo-phosphamide against V79 cells in vitro. Furthermore, the *in vivo* anticancer activity was just as good or better than that of cyclophosphamide against wild type P388 cells and was substantially superior to cyclophosphamide against cyclophosphamide-resistant P388 cell line.

Numerous structural modifications on cyclophosphamide (CP) have been made in order to understand its mechanism of action and to increase its antitumor efficacy (Zon, 1982; Borch and Canute, 1991; Stec, 1982; Ludeman et al., 1986; Boyd et al., 1980). Among the analogs studied, mafosfamide (ASTA Z7557), originally introduced as a chemically "stable" derivative of 4-



Scheme 2. *N*-substituted CP

OH-CP, contains a thiol, viz., MESNA (2-mercaptoethanesulfonate) at C_4 on the oxazaphosphorine ring. The antitumor activity of mafosfamide is comparable to CP, but it shows less myelo and urotoxicity than CP (Pohl, 1983; Pohl *et al.*, 1984). However, mafosfamide causes a severe local toxicity presumably due to its rapid hydrolysis to 4-OH-CP (Bruntsch *et al.*, 1985; Abele *et al.*, 1986; Kwon *et al.*, 1987). The presence of alkyl substituent at N_3 in the oxazaphosphorine ring was found to stabilize *N*-substituted 4-(alkylthio)cyclophosphamide derivatives from "spontaneous" decomposition (Scheme 2).

Based on this finding, several *N*-methyl-4-alkylthiocyclophosphamide derivatives, viz., *N*-methyl-4-(*n*-propylthio)cyclophosphamide, *N*-methyl-4-(diethyldithiocarbamoyl) cyclophosphamide and *N*-methyl-mafosfamide were synthesized and examined as chemically stable, bio-oxidative prodrugs of 4-hydroxycyclophosphamide (4-OH CP) (Moon *et al.*, 1995; Moon and Kwon, 1998). The *N*-methyl-4-thiocyclophosphamide derivatives were expected to undergo *N*-demethylation, initially to produce 4-thiocyclophosphamides, which in turn should readily generate 4-OH-CP/Aldo and the corresponding thiol (Scheme 2). It was also hypothesized that the thiol liberated should be more effective in intercepting acrolein, the unwanted toxic byproduct of further 4-OH CP/Aldo activation than the systemically administered thiol. All of the derivatives were stable in aqueous buffer (pH 7.4, 37°C) and underwent *N*-demethylation in a time dependent manner when incubated with rat hepatic microsomes, which resulted in formation of alkylating species. *In vitro* cytotoxicity profile of *N*-methyl-4-(*n*-propylthio)cyclophosphamide and *N*-methyl-mafosfamide was similar to that of cyclophosphamide (CP) against mouse embryo Balb/c 3T3 cells as well as against a panel of human tumor cell lines. Interestingly, *N*-methyl-4-(diethyldithiocarbamoyl)cyclophosphamide was considerably more cytotoxic to 3T3 cells and certain selected human tumor cell lines. Preliminary antitumor screening against L1210 leukemia in mice suggests that these prodrugs, especially *N*-methyl-4-(diethyldithiocarbamoyl)cyclophosphamide, have significant antitumor activity *in vivo*.

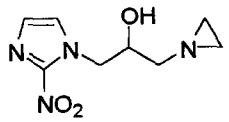
Glutathione-*S*-transferase (GST) levels have frequently been found to be elevated in manny tumors, e.g., lung, colon, and stomach cancers relative to normal surrounding tissue (Tew and Clapper, 1987). On the basis of this finding, several compounds were synthesized, which are designed to release a cytotoxic phosphoramidate species by the action of GSTs (Lyttle *et al.*, 1994). The cytotoxicity with MCF-7 breast cancer cells showed enhanced sensitivity toward cells transfected to overexpress GST.

A correlation between curative response to aniline mustard in tumor-bearing mice and glucuronidase activity in the tumors provided the evidence for a role of β-glucuronidase in selective activation of prodrugs (Connors and Whisson, 1966). Aniline mustard underwent oxidation to *p*-hydroxyaniline mustard, which in turn further metabolized to the less toxic *O*-glucuronide. It was proposed that the glucuronide regenerate the toxic *p*-hydroxyaniline musard in tumors with high β-glucurinidase activity (Connors *et al.*, 1973). Clinical response to aniline mustard, however, has been mixed despite high levels of the enzyme (Young *et al.*, 1976).

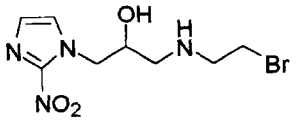
Bioreductive Prodrugs

Solid tumors contain areas of low oxygen tension (hypoxia) generally thought to arise in solid tumors due to poor and disorganized blood supply (Brown, 1997). These tumor cells are particularly resistant to radiation and chemotherapy. A number of factors including cellular heterogeneity and physiological properties such as inadequate blood flow are involved in the lack of responsiveness (Warren, 1978; Poste, 1986). While there may be few useful kinetic and/or biochemical differences between solid tumor cells and normal cells that can be exploited, there are important microenvironmental properties unique to solid tumors, e.g., localized hypoxia, nutrient deprivation, and low pH (Kennedy *et al.*, 1980).

There has been considerable interest in designing drugs selective for hypoxic environments (Denny and Wilson, 1986; Stratford *et al.*, 1986; Sartorelli, 1988; Wilson *et al.*, 1989a and 1989b; Denny *et al.*, 1990). A comprehensive review on development of hypoxia-selective radiosensitizers has recently appeared (Suto, 1991). Much of the work has been centered on nitroheterocyclics that utilize nitroreductase enzyme systems for their activation. In general, nitroimidazoles, especially the bifunctional nitroimidazoles bearing alkylating moieties, e.g. RSU 1069 and RB 6145, were found to be more effective radiosensitizers than the other nitroarenes tested (Fielden



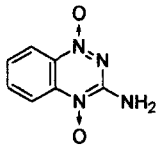
RSU 1069



RB 6145

et al., 1992).

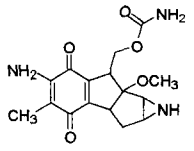
In an effort to increase the molar efficiency of nitroimidazoles, DNA-targeted radiosensitizers containing nitroimidazoles linked to intercalating moieties such as phenanthrene (NLP-1) and acridine (NLA-1) have appeared (Denny *et al.*, 1992; Cowan *et al.*, 1992). Another interesting series of hypoxia-selective cytotoxins is benzotriazine di-*N*-oxides. The lead compound, tirapazamine (SR 4233; WIN 59075) has shown particularly impressive hypoxic cell specificity (Walton and Workman, 1990).



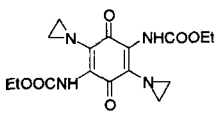
Tirapazamine (SR 4233)

Tirapazamine has shown impressive anticancer activity when combined with other chemotherapeutic agents (Lartigau and Guichard, 1996) or with irradiation (Shibata et al., 1996). Some of the second-generation 1,2,4-benzotriazine 1,4-di-N-oxides have been reported to show better hypoxic toxicity profiles than tirapazamine in vitro (Michinto *et al.*, 1992).

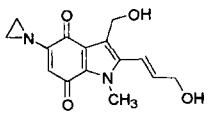
Mitomycin C is in widespread clinical use and is considered as the prototype bioreductive alkylating agent (Sartorelli, 1988). Mitomycin C, following reduction of the quinone moiety, produces two electrophilic centers via the loss of the carbamate group and the activation of the aziridine ring. This results in intra- and/or interstrand cross-linking of DNA (Tomaz *et al.*, 1987). Similar bioactivation is thought to take place with structurally related compounds such as indolquinone EO9 (Oostveen and Speckamp, 1987) and diaziridinylbenzoquinone [diazoquone; AZQ] (Smigiero and Kohn, 1984).



Mitomycin C



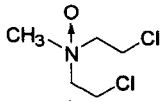
Diaziquone (AZQ)



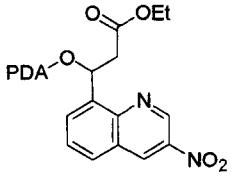
EO9

Nitrophenyl mustard derivatives have also been proposed as bioreductive alkylating agents (Denny and Wilson, 1986). Among the series of aniline mustards with a wide range of electron-withdrawing and -donating substituents, only 4-nitro aniline mustard showed some hypoxia-selectivity (Palmer et al., 1990a). Nitro analogs of chlorambucil, which contain weakly-acidic side chains that were presumed to have the potential to drive selective intracellular accumulation of drugs in the low pH environment of solid tumor tissue, were evaluated (Palmer *et al.*, 1990b), but fail to show significant hypoxia-selective cytotoxicity. A more recent study on a series of water-soluble nitroaniline mustards showed (Palmer *et al.*, 1992) that some of the 2,4-dinitro derivatives may be promising hypoxia-selective cytotoxins, although the mechanism, from which the cytotoxicity is derived, does not appear to involve the typical DNA cross-linking of mustard.

Nitromin (nitrogen mustard *N*-oxide) has been used with some success for the treatment of Yoshida ascites sarcomas in rats (Aiko *et al*, 1952). Recent studies showed that nitromin serves as a bioreductive prodrug of nitrogen mustard (Connors, 1983; White *et al.*, 1989).



Nitromin



Nitroquinoline PDA

A novel nitroquinoline containing phosphoramide mustard (PDA) moiety, the "ultimate" cytotoxic metabolite of cyclophosphamide, was synthesized as a prototype hypoxia-selective alkylating agent (Firestone *et al.*, 1991). This phosphoramidate was 11-fold more cytotoxic to HT-29 human colon carcinoma cells under hypoxic compared to aerobic conditions.

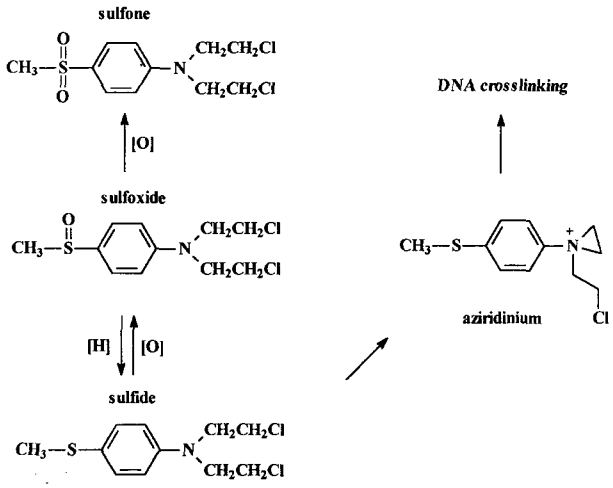
Bioreductive activation of these hypoxia-selective cytotoxins can be catalyzed by a number of enzymes such as cytochrome P-450, cytochrome P-450 reductase, DT-diaphorase (quinone-oxidoreductase), xanthine oxidase, and aldehyde oxidase (Walton *et al.*, 1989; Workman and Walton, 1991). It appears that several different enzymes participate to different extents with the various bioreductive agents (Workman, 1992). Workman suggests a distinct possibility that one can design a successful bioreductive drug possessing a promiscuous relationship with several reductases or tailored for a particular enzyme that might be hyper-expressed in a particular tumor.

Sulfoxides are known to be susceptible to bioreduction and the reduction of sulfoxides by mammalian tissues is a complex process which may involve both soluble and membrane-bound enzyme systems (Renwick, 1989). One of the earliest reports on the hepatic reduction of sulfoxides was that of 4,4'-diaminodiphenylsulfoxide which was readily reduced by 10,000 g supernatant fractions of rat liver in the presence of NADPH but not NADH (Mazel *et al.*, 1969). The amino acid methionine, in its oxidized form cannot be utilized as such for protein synthesis; this suggested that the methionine sulfoxide (Met SO) must be reduced to methionine *in vivo* (Sourkes and Tano, 1953). *In vitro* reduc-

tion of Met SO by rat liver and kidney showed that activity was found mainly in the cytosol of liver and was enhanced by NADH (Aymard et al., 1979). Sulindac, a well-known non-steroidal anti-inflammatory drug, undergoes two major biotransformations: reversible reduction of the sulfoxide compound (parent drug) to the sulfide metabolite and irreversible oxidation to the sulfone metabolite (Duggan *et al.*, 1978). The oxidation to the sulfone metabolite is the dominant process under normal physiological condition; however, the reduction process becomes significant under anaerobic conditions (Davis and Guenthner, 1985). The enzyme systems involved in the reduction include thioredoxin and thioredoxin reductase present in a variety of mammalian tissues (Anders *et al.*, 1980) as well as liver aldehyde oxidase and xanthine oxidase in the presence of an electron donor (Tatsumi *et al.*, 1982; Kitamura and Tatsumi, 1983).

These literature data suggested that properly designed sulfoxide compounds, which can be converted to and/or generate alkylating species upon bioreduction, might serve as useful hypoxia-selective anticancer agents. *p*-(Methylsulfinyl)phenyl nitrogen mustard {4-[bis(2-chloroethyl)amino]-1-(methylsulfinyl)benzene}, was examined as a prototype bioreductive prodrug of *p*-(methylthio) phenyl nitrogen mustard (Kwon *et al.*, 1992). It was proposed that the sulfoxide prodrug should be chemically stable because of the electron withdrawing effect by the sulfoxide moiety, hence the nitrogen lone pair of the nitrogen moiety is not available to readily form the reactive aziridinium species. However, upon reduction in the hypoxic environments of tumor cells, the sulfoxide is expected to generate the reactive sulfide (Scheme 3). Under the aerobic conditions found in normal cells, the sulfoxide is expected to be metabolized to the more chemically stable sulfone metabolite.

The relative chemical reactivity of these compounds showed that the sulfide was the most reactive, followed by the sulfoxide and the sulfone. The relative cytotoxicity profile of these compounds against 3T3 cell line correlated well in a qualitative manner with their chemical reactivity data; the cytotoxicity decreased in the order of the sulfide, sulfoxide, and sulfone. *In vitro* study with the rat S-9 fraction showed that the sulfoxide prodrug was bioreduced to the sulfide more significantly under anaerobic condition in the presence of NADPH-generating system. This suggested a possible involvement of NADPH-dependent cytochrome P-450 reductase, which has been shown to catalyze the reduction of nitromin to nitrogen mustard (White *et al.*, 1989). These results provided the first evidence supporting the concept of enhanced bioreduction of the sulfoxide to the sulfide under hypoxic condition. The sulfoxide prodrug showed some hypoxia-selective cytotoxicity against the Chinese hamster ovary (CHO) cell line as well (Kwon *et al.*, 1992). When the sulfoxide was incubated with CHO cells under



Scheme 3. A model of prototype bioreductive prodrug

hypoxic conditions there was a 4-fold enhancement of cytotoxicity as compared to aerobic conditions, on the basis of the IC_{50} values of the cell survivals. The hypoxia selectivity was however less substantial at higher drug concentrations with the enhancement of ca. 2 fold at the level of IC_{90} or greater.

It has been reported that the enzyme systems involved in sulfoxide reduction apparently show different substrate specificity (Lee and Renwick, 1995; Renwick, 1989). To further understand the relationship between the hypoxia-selectivity and the sulfoxide structures, selected model sulfoxide compounds were studied for their metabolism with emphasis on bioreduction. These included methylphenyl sulfoxide, diphenyl sulfoxide and dibenzothiophene-5-one. The chemical reduction potential data of the model sulfoxides indicated that dibenzothiophene-5-one was the most easily reduced, followed by diphenylsulfoxide and methylphenylsulfoxide. On the other hand, diphenylsulfoxide was the most easily bioreduced by the rat S-9 fraction, followed by methylphenylsulfoxide and dibenzothiophene-5-one. Dibenzothiophene-5-one was poorly reduced by aldehyde oxidase despite its low chemical redox potential. Further studies of diphenylsulfoxide with the rat S-9 fractions showed that the formation of diphenylsulfone was observed only in the presence of NADPH under aerobic condition, suggesting that NADPH-dependent enzymes were responsible for the oxidation. Under anaerobic condition, diphenylsulfoxide was reduced to diphenylsulfide in the presence of benzaldehyde, 2-hydroxypyrimidine, or acetaldehyde. On the other hand, NADPH did not appear to be involved in catalyzing this reduction. In addition, the reduction was inhibited by menadione, a specific aldehyde oxidase inhibitor. All these findings suggested that liver aldehyde oxidase, not other NADPH dependent enzymes, was the enzyme involved in the reduction of

diphenylsulfoxide under anaerobic condition. Under aerobic conditions, diphenylsulfoxide was not reduced to the corresponding sulfide in the presence of benzaldehyde. This was consistent with the literature observation (Yoshihara and Tatusumi, 1985) that sulfoxide reduction by aldehyde oxidase is inhibited by oxygen.

Subsequently, 1-[bis(2-chloroethyl)amino]-4-[{4-[bis(2-chloroethyl)amino]phenyl}sulfinyl]benzene, a diphenylsulfoxide-containing nitrogen mustard, was synthesized and its hypoxia-selective cytotoxicity was examined against V79 Chinese hamster lung fibroblasts (Kwon, 1999b). The preliminary results showed that this compound was>10-fold more toxic to V79 cells under hypoxic conditions compared to aerobic conditions, on the basis of the slopes of the survival curves. A comparison cytotoxicity test against V79 cells with the previously reported compound, 4-[bis(2-chloroethyl)amino]-1-(methylsulfinyl)benzene, an alkylphenylsulfoxide-containing nitrogen mustard, suggested that the substantial hypoxia-selective toxicity exhibited by 1-[bis(2-chloroethyl)amino]-4-[{4-[bis(2-chloroethyl)amino] phenyl}sulfinyl]benzene was not due to some unique feature of V79 cells, but more likely to the modification of the structure that led to more favorable bioactivation by sulfoxide reductase(s). Furthermore, 1-[bis(2-chloroethyl) amino]-4-[{4-[bis(2-chloroethyl)amino]phenyl}sulfinyl] benzene displayed 7-fold further enhanced hypoxia-selective cytotoxicity against V79 cells in the presence of benzaldehyde. Benzaldehyde is a well known electron donor for aldehyde oxidase. The above finding suggests that V79 cells contain aldehyde oxidase and a significantly enhanced reduction of the sulfoxide to the cytotoxic sulfide takes place in the presence of a suitable electron donor for aldehyde oxidase. These preliminary data may warrant further studies in the exploitation of the sulfoxide-containing mustard derivatives as hypoxia-selective anticancer agents.

In summary, a number of different prodrug strategies have been applied to improve target specificity against cancer cells and/or to alleviate toxicity associated with the use of anticancer drugs. The search continues for drugs that can kill cancer cells selectively by exploiting unique biochemical or physiological mechanisms of cancer cells. Cancer cells are usually heterogeneous and this may explain some of the disappointing clinical results of the prodrugs based on elevated enzyme levels. Hypoxia, the unique feature of solid tumors, however, appears to be an attractive target of exploitation in the design of successful bioreductive prodrugs as evidenced by tirapazamine and others.

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