Biochemical Pharmacology, Vol. 34, No. 19, pp. 3465–3471, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

ALDEHYDE DEHYDROGENASE ACTIVITY AS THE BASIS FOR THE RELATIVE INSENSITIVITY OF MURINE PLURIPOTENT HEMATOPOIETIC STEM CELLS TO OXAZAPHOSPHORINES*

FRED R. KOHN and NORMAN E. SLADEK†
Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

(*Received* 9 *October* 1984; *accepted* 28 *February* 1985)

Abstract—The *ex vivo* sensitivity of murine pluripotent hematopoietic stem cells (CFU-S) and myeloid progenitor cells (CFU-GM) to 4-hydroperoxycyclophosphamide, ASTA Z 7557, phosphoramide mustard, acrolein, melphalan, and *cis*-platinum was determined in the absence and presence of known (disulfiram, diethyldithiocarbamate, cyanamide) or suspected [ethylphenyl(2-formylethyl)phosphinate] inhibitors of aldehyde dehydrogenase activity. As compared to CFU-GM, CFU-S were less sensitive to the oxazaphosphorine agents, 4-hydroperoxycyclophosphamide and ASTA Z 7557. The two cell populations were approximately equisensitive to acrolein as well as to the non-oxazaphosphorine cross-linking agents, phosphoramide mustard, melphalan and *cis*-platinum. All four inhibitors of aldehyde dehydrogenase activity potentiated the cytotoxic action of the oxazaphosphorines toward CFU-S; they did not potentiate the cytotoxic action of acrolein or the non-oxazaphosphorines toward these cells. The inhibitors did not potentiate the cytotoxic action of the oxazaphosphorines, non-oxazaphosphorines, or acrolein toward CFU-GM. Pyridoxal, a substrate for aldehyde oxidase, did not potentiate the cytotoxic action of oxazaphosphorines toward CFU-S. Cellular NAD-linked aldehyde dehydrogenases are known to catalyze the oxidation of the major transport form of cyclophosphamide, 4-hydroxycyclophosphamide/aldophosphamide, to an inactive metabolite, carboxyphosphamide. Our observations suggest that (1) aldehyde dehydrogenase activity is an important determinant of the sensitivity of a cell population to the oxazaphosphorines, (2) CFU-GM lack the relevant aldehyde dehydrogenase activity, and (3) the phenotypic basis for the relative insensitivity of CFU-S to oxazaphosphorines is the aldehyde dehydrogenase activity contained by these cells.

The 2-chloroethylamido-oxazaphosphorine, cyclophosphamide, has been used extensively as an antineoplastic and immunosuppressive agent because of its well established therapeutic efficacy and relatively favorable margin of safety. Cyclophosphamide is not active in parent form. It is first hydroxylated via a reaction catalyzed by hepatic mixed-function oxidases. The product, 4-hydroxycyclophosphamide, can give rise to aldophosphamide which, in turn, can give rise to acrolein and the active DNA cross-linking metabolite, phosphoramide mustard. Aldophosphamide can also be oxidized to an inactive product, carboxyphosphamide, via a reaction catalyzed by cellular NAD-linked aldehyde dehydrogenases [1–8]. Using acetaldehyde, butyraldehyde, and benzaldehyde as substrates, several laboratories have identified aldehyde dehydrogenase activity in varying amounts in a wide variety of tissues [6, 9].

The biochemical basis for the relatively good therapeutic index of cyclophosphamide has not been elucidated but apparently resides with the 4-hydroxy/aldehyde intermediates rather than with the reactive mustard [10–13]. It has been proposed that the selective action of cyclophosphamide depends, at least in part, on the balance between conversion of aldophosphamide to active and inactive metabolites in sensitive and insensitive cells [3–5, 14–16]. An inverse correlation between aldehyde dehydrogenase activity and sensitivity to oxazaphosphorines has been found in a variety of cultured murine and human neoplastic cells [8, 17, 18].

The relatively favorable margin of safety exhibited by cyclophosphamide may be due, at least in part, to the relative hematopoietic stem cell sparing effect [19, 20] of the drug since bone marrow toxicity is, in general, the major limiting factor in the chemotherapy of neoplastic diseases. The present investigation was undertaken to quantify the sensitivity of murine pluripotent hematopoietic stem cells (CFU-S) and myeloid progenitor cells (CFU-GM) to oxazaphosphorine and non-oxazaphosphorine agents *ex vivo* and to examine the influence of inhibitors of aldehyde dehydrogenase activity on the sensitivity of these hematopoietic cell subpopulations to oxazaphosphorines.

* Supported by USPHS Grant CA 21737. A description of parts of this investigation has appeared in abstract form [F. R. Kohn and N. E. Sladek, *Proc. Am. Ass. Cancer Res.* **25**, 689 (1984)].

† To whom request for reprints should be addressed, at the University of Minnesota, Department of Pharmacology, 3-260 Millard Hall, 435 Delaware St., S.E., Minneapolis, MN 55455.

MATERIALS AND METHODS

Materials. ASTA Z 7557‡§, 4-hydroperoxycyclophosphamide, phosphoramide mustard·cyclohexylamine, melphalan HCl, and EPP were supplied by Dr. P. Hilgard (Asta-Werke AG, Bielefeld, Federal Republic of Germany), Dr. A. Takamizawa (Shionogi & Co., Fukushimaku, Osaka, Japan), Mr. L. H. Kedda (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD), Dr. G. M. Lyon, Jr. (Burroughs Wellcome & Co., Research Triangle Park, NC), and Dr. L. A. Cates (College of Pharmacy, University of Houston, Houston, TX) respectively. Acrolein was purchased from the Aldrich Chemical Co., Milwaukee, WI. *Cis*-platinum II diamine dichloride, disulfiram, DDTC, cyanamide, pyridoxal hydrochloride and *l*-asparagine were purchased from the Sigma Chemical Co., St. Louis, MO. Methylcellulose (A4M premium grade, 4000 cps) was supplied by the Dow Chemical Co., Midland, MI. Powdered RPMI 1640 medium, CMRL 1066 medium, fetal bovine serum, horse serum and gentamicin were purchased from the GIBCO Laboratories, Grand Island, NY.

All drugs were dissolved in drug-exposure medium (described below) with the exception of disulfiram which was dissolved in 95% ethanol. All drug solutions, except for those containing acrolein or disulfiram, were sterilized by passage through 0.22 μm Millipore filters; all were used within 30 min of preparation and were kept on ice prior to their use. At the concentrations used, ethanol was not cytotoxic to CFU-S or CFU-GM nor did it alter the sensitivity of CFU-S or CFU-GM to the oxazaphosphorines.

Drug-exposure medium contained $CaCl_2$ (100 mg/l), KCl (200 mg/l), $MgCl_2 \cdot 6H_2O$ (100 mg/l), KH_2PO_4 (42 mg/l), $Na_2HPO_4 \cdot 7H_2O$ (453 mg/l) and NaCl (8614 mg/l) dissolved in triple-distilled water. The pH was adjusted to 7.4 prior to passage through a 0.22 μm Millipore filter.

Animals. BALB/c mice were bred and raised at the University of Minnesota Mouse Colony. Male mice, aged 12–16 weeks, were used in all experiments. Animals were housed in plastic cages with filtered lids and received standard laboratory food and water *ad lib.* Irradiated mice received water, supplemented with oxytetracycline-neomycin, *ad lib.* throughout the course of the experiment.

Preparation of murine bone marrow cell suspensions and drug exposure. Bone marrow cells were flushed from femurs of two to four BALB/c mice with drug-exposure medium using a tuberculin syringe fitted with a 27-gauge needle. Following pooling of the cells in additional medium, the concentration of viable nucleated cells was determined with the aid of a hemacytometer using the method of trypan blue exclusion. The cell suspension was then diluted to the desired viable cell concentration with additional medium, and portions were transferred to 15-ml conical centrifuge tubes.

To determine the sensitivity of murine CFU-S or CFU-GM to various cytotoxic agents, drug-free medium (control) or freshly prepared drug solutions were first added to the cell suspensions (4×10^5 nucleated cells/ml in a total volume of 5 ml). The suspensions were then incubated for 30 min at 37°. Immediately following drug exposure, cells were harvested by centrifugation (5 min at 300 *g*), and subsequently resuspended in appropriate drug-free medium (RPMI 1640 medium for the CFU-S assay and CMRL 1066 medium for the CFU-GM assay). The cell suspensions were then assayed for CFU-S or CFU-GM as described below.

To determine the effect of inhibitors of aldehyde dehydrogenase activity on the sensitivity of murine CFU-S or CFU-GM to various cytotoxic agents, marrow cell suspensions were first incubated for 60 min at 37° in the presence or absence of the inhibitor. Drug-free medium or cytotoxic agent in solution was then added, and incubation was continued at 37° for an additional 30 min. Immediately following drug exposure, cells were harvested and resuspended in drug-free medium as described above, and the resultant cell suspensions were assayed for CFU-S or CFU-GM.

CFU-S assay. The assay was based on the method of Till and McCulloch [21]. Drug-treated or vehicle-treated marrow cells in suspension (8×10^4 nucleated cells in a volume of 0.2 ml) were injected i.v. into four or five irradiated BALB/c mice; the mice had received 750 rad total body irradiation (Mark I ^{137}Cs irradiator) on the previous day. On day 8 following the marrow transplant, mice were killed by cervical dislocation, and their spleens were excised and fixed in Bouin's solution. Macroscopic spleen colonies were counted with the aid of a dissecting microscope. Control colony formation ranged from 20 to 26 colonies/8×10^4 nucleated cells injected.

CFU-GM assay. A modification of the method of Worton *et al.* [22] was used. Drug-treated or vehicle-treated marrow cells in suspension were added to CMRL 1066 medium containing methylcellulose (0.6%, w/v), fetal bovine serum (10%, v/v), horse serum (5%, v/v), L-929 cell conditioned medium (20%, v/v), *l*-asparagine (20 μg/ml) and gentamicin (50 μg/ml). Portions (1 ml) of the resultant suspension (1×10^5 nucleated cells) were plated in triplicate in 35 mm Falcon petri dishes. The seeded dishes were then incubated at 37° in a fully humidified atmosphere containing 10% CO_2. On day 7 following plating, granulocyte-macrophage colonies (>50 cells) were counted with the aid of an inverted microscope. Control colony formation ranged from 90 to 190 colonies/10^5 nucleated cells plated.

RESULTS

Initial experiments were designed to quantify the sensitivity of murine bone marrow CFU-S and CFU-

‡ Abbreviations: ASTA Z 7557, 2-[bis-(2-chloroethyl)-amino]-4-(2-sulfoethylthio)-tetrahydro-2*H*-1,3,2-oxaza-oxazaphosphorine-2-oxide cyclohexylamine salt; EPP, ethylphenyl (2-formylethyl)phosphinate; DDTC, diethyldithiocarbamate; and IC_{50}, concentration of drug required to produce a 50% inhibition of colony formation.

§ ASTA Z 7557 and 4-hydroperoxycyclophosphamide are relatively stable precursors of 4-hydroxycyclophosphamide. Both precursors rapidly and spontaneously (without benefit of enzymatic involvement) give rise to the 4-hydroxy intermediate under physiologic conditions.

Table 1. Sensitivity of murine CFU-S and CFU-GM to cytotoxic agents *ex vivo*: IC_{50} values.*

Agent	IC_{50} (95% C.I.), μM	
	CFU-S	CFU-GM
ASTA Z 7557	55 (51–59)	17 (16–19)
4-Hydroperoxycyclophosphamide	35 (28–43)	10 (9–10)
Phosphoramide mustard	60 (56–65)	73 (70–76)
Acrolein	2.1 (1.8–2.4)	1.5 (1.4–1.6)
Melphalan	0.32 (0.29–0.36)	0.23 (0.22–0.25)
Cis-platinum	22 (19–26)	25 (23–28)

* BALB/c bone marrow cells were incubated for 30 min at 37° in the absence or presence of cytotoxic agent. The cells were then harvested, resuspended in drug-free medium, and assayed for CFU-S or CFU-GM as described in Materials and Methods. Data from one or two experiments were plotted as in Fig. 1, and IC_{50} values and 95% confidence intervals were generated from regression lines with computer assistance using the method of Tallarida and Murray [23].

GM to oxazaphosphorines (4-hydroperoxycyclophosphamide, ASTA Z 7557), acrolein, and non-oxazaphosphorine cross-linking agents (phosphoramide mustard, melphalan, *cis*-platinum) *ex vivo* (Table 1). Examples of the log-linear concentration–response curves used to generate IC_{50} values are presented in Fig. 1. CFU-S were 3.2 and 3.5 times less sensitive to ASTA Z 7557 and 4-hydroperoxycyclophosphamide, respectively, than were CFU-GM. A similar difference in sensitivity to acrolein and the non-oxazaphosphorine agents was not observed.

In the next series of experiments, known (disulfiram, DDTC, cyanamide) or suspected (EPP) inhibitors of aldehyde dehydrogenase activity were used to determine the effect of enzyme inhibition on the sensitivity of CFU-S and CFU-GM to ASTA Z 7557, 4-hydroperoxycyclophosphamide, phosphoramide mustard, and acrolein (Fig. 2). Inhibitor concentrations chosen were those that had no effect on CFU-S and minimal effect on CFU-GM. None of the inhibitors potentiated the cytotoxic action of ASTA Z 7557 or 4-hydroperoxycyclophosphamide toward CFU-GM; all four inhibitors potentiated the

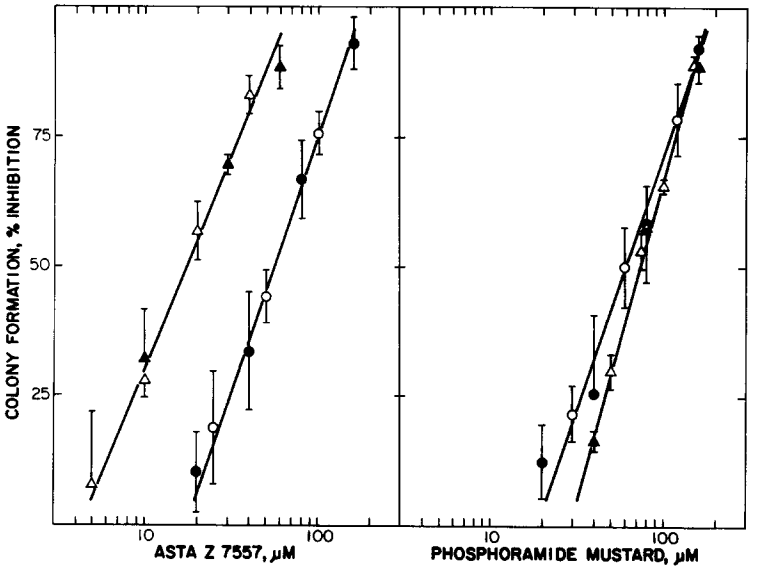

Fig. 1. Sensitivity of murine CFU-S and CFU-GM to ASTA Z 7557 and phosphoramide mustard *ex vivo*. BALB/c bone marrow cells were incubated for 30 min at 37° in the absence or presence of cytotoxic agent. The cells were then harvested, resuspended in drug-free medium, and assayed for CFU-S (●, ○) or CFU-GM (▲, △) as described in Materials and Methods. Points: mean ± S.D. of observations made in four or five mice (CFU-S) or triplicate plates (CFU-GM). Lines were generated with computer assistance using regression analysis. Results of two separate experiments (open and closed symbols) are shown for each line.

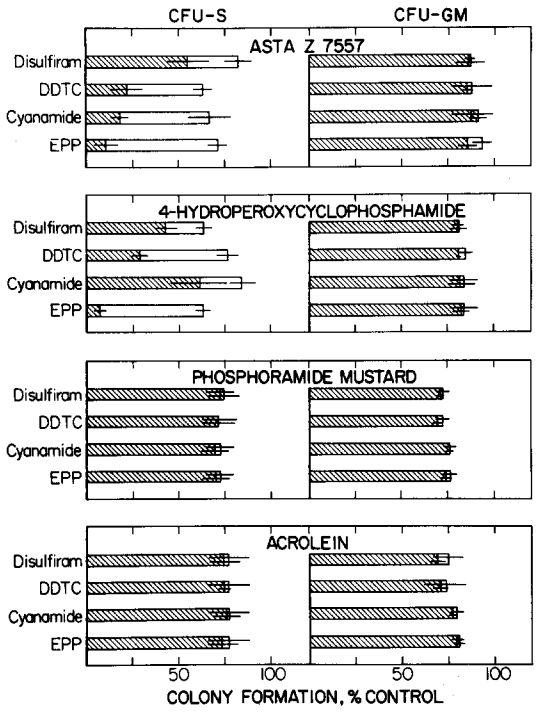

Fig. 2. Sensitivity of murine CFU-S and CFU-GM to fixed concentrations of cytotoxic agents in the absence and presence of known or suspected inhibitors of aldehyde dehydrogenase activity. BALB/c bone marrow cells were incubated for 60 min at 37° in the absence (open bars) or presence (hatched bars) of disulfiram (0.5 μM), DDTC (100 μM), cyanamide (60 μM) or EPP (100 μM). Vehicle or cytotoxic agent was then added and incubation was continued at 37° for an additional 30 min. Concentrations of cytotoxic agents used in the CFU-S and CFU-GM experiments, respectively, were ASTA Z 7557 (20 μM, 5 μM), 4-hydroperoxycyclophosphamide (10 μM, 4 μM), phosphoramide mustard (30 μM, 40 μM) and acrolein (1 μM, 0.8 μM). Following incubation, the cells were harvested, resuspended in drug-free medium, and assayed for CFU-S or CFU-GM as described in Materials and Methods. Results are expressed as percent of the relevant control. None of the four inhibitors depressed colony formation in the CFU-S assay. In the CFU-GM assay, colony formation in the presence of inhibitors, expressed as percent of control (no inhibitor) colony formation, was 65 ± 5, 57 ± 4, 90 ± 4 and 91 ± 4 (mean ± S.E. of observations made in triplicate plates in each of four experiments) for disulfiram, DDTC, cyanamide and EPP respectively. Bars: mean ± S.D. for observations made in four or five mice (CFU-S) or triplicate plates (CFU-GM).

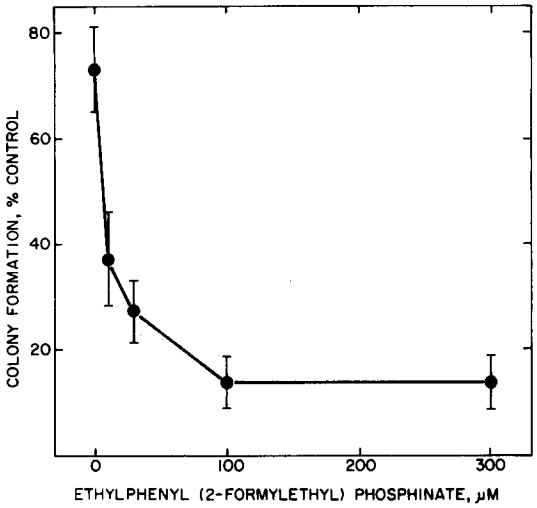

Fig. 3. Sensitivity of murine CFU-S to a fixed concentration of ASTA Z 7557 in the presence of increasing concentrations of EPP. BALB/c bone marrow cells were incubated for 60 min at 37° in the absence or presence of EPP. Vehicle or ASTA Z 7557 (20 μM) was then added, and incubation was continued at 37° for an additional 30 min. Following incubation, the cells were harvested, resuspended in drug-free medium, and assayed for CFU-S as described in Materials and Methods. Results are expressed as percent of the relevant control. EPP was not cytotoxic to CFU-S at any of the concentrations tested. Points: mean ± S.D. of observations made in four or five mice.

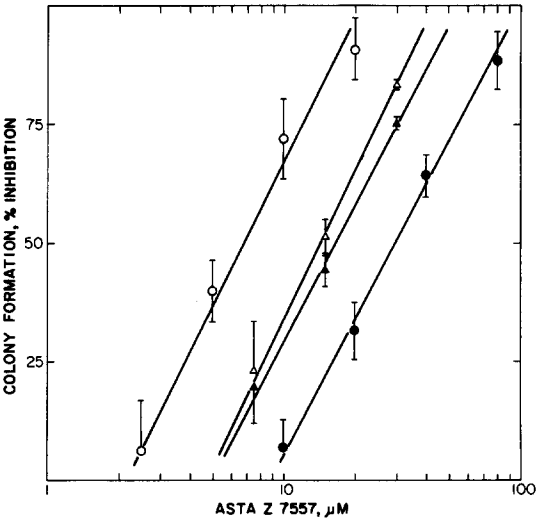

Fig. 4. Sensitivity of murine CFU-S and CFU-GM to ASTA Z 7557 in the absence and presence of a fixed concentration of EPP. BALB/c bone marrow cells were incubated for 60 min at 37° in the absence (closed symbols) or presence (open symbols) of 100 μM EPP. Vehicle or ASTA Z 7557 was then added and incubation was continued at 37° for an additional 30 min. Following incubation, the cells were harvested, resuspended in drug-free medium, and assayed for CFU-S (●, ○) or CFU-GM (▲, △) as described in Materials and Methods. Results are expressed as percent of the relevant control. Points: mean ± S.D. of observations made in four or five mice (CFU-S) or triplicate plates (CFU-GM).

cytotoxic action of these agents toward CFU-S. None of the four inhibitors potentiated the cytotoxic action of phosphoramide mustard or acrolein toward CFU-S or CFU-GM. Furthermore, EPP (100 μM) or DDTC (300 μM) did not potentiate the cytotoxic action of melphalan or *cis*-platinum toward CFU-S (data not presented).

Pyridoxal is a known substrate for aldehyde oxidase [24] and is therefore expected to competively inhibit the oxidation of other aldehydes by this enzyme. At a concentration of 300 μM, it did not

potentiate the cytotoxic action of ASTA Z 7557 toward CFU-S or CFU-GM (data not presented), suggesting that aldehyde oxidase activity is not a determinant of the sensitivity of CFU-S or CFU-GM to oxazaphosphorines.

The ability of EPP to potentiate the cytotoxic action of ASTA Z 7557 toward CFU-S was examined in more detail. In the first experiment, bone marrow cells in suspension were exposed to a fixed concentration of ASTA Z 7557 in the presence of increasing concentrations of EPP (Fig. 3). EPP effected a concentration-dependent potentiation of the cytotoxic action of ASTA Z 7557 with maximum potentiation occurring when concentrations of 100 μM and greater were used. In a second experiment, bone marrow cells were exposed to various concentrations of ASTA Z 7557 in the presence and absence of a fixed concentration of EPP (Fig. 4). CFU-S were 4.5 times more sensitive to ASTA Z 7557 in the presence of EPP; IC_{50} values were 6.7 and 30 μM in the presence and absence of EPP respectively. As before, the sensitivity of CFU-GM to ASTA Z 7557 in the presence of EPP was not significantly different from that determined in the absence of EPP; IC_{50} values were 14 and 16 μM respectively. It should be noted that the sensitivity of CFU-S to ASTA Z 7557 was increased significantly following a 60-min preincubation at 37° in drug-free medium as compared to exposure to the drug without preincubation; IC_{50} values were 30 and 55 μM respectively (Fig. 4 and Table 1). On the other hand, the sensitivity of CFU-GM to ASTA Z 7557 was not affected by a 60-min preincubation; IC_{50} values were 16 and 17 μM with and without preincubation respectively.

DISCUSSION

Murine CFU-S, as compared to CFU-GM, were found to be relatively insensitive to the oxazaphosphorines, ASTA Z 7557 and 4-hydroperoxycyclophosphamide. A similar differential sensitivity to acrolein and to the non-oxazaphosphorine cross-linking agents, phosphoramide mustard, melphalan and *cis*-platinum, was not observed. Given that ASTA Z 7557, 4-hydroperoxycyclophosphamide, phosphoramide mustard, melphalan, and *cis*-platinum produce their cytotoxic action by the same mechanism, CFU-S insensitivity to oxazaphosphorines must be due to factors that influence the cytotoxic action of oxazaphosphorines specifically, e.g. cellular uptake or biotransformation [1–8, 25–30], and not to factors which would affect the cytotoxic action of all cross-linking agents, e.g. DNA repair. This is not to say that such nonspecific factors would not be operative, only that they would not be responsible for the observed differential sensitivity.

The rate of conversion of 4-hydroxycyclophosphamide/aldophosphamide to the inactive metabolite, carboxyphosphamide, a reaction catalyzed by cellular NAD-linked aldehyde dehydrogenases, has been proposed to be a major determinant of the sensitivity of neoplastic and normal cells to oxazaphosphorines [3–5, 8, 14–18]. Target cells containing a relatively greater aldehyde dehydrogenase activity would be relatively insensitive to oxazaphosphorines since less 4-hydroxycyclophosphamide/aldophosphamide would be available for conversion to cytotoxic metabolites in these cells. Hilton and Cohen [8] have observed that cyclophosphamide-resistant murine L1210 cells contain greater aldehyde dehydrogenase activity than do wild-type, cyclophosphamide-sensitive L1210 cells. Inclusion of the aldehyde dehydrogenase inhibitor disulfiram in the incubation medium restored the sensitivity of the resistant cells to 4-hydroxycyclophosphamide but had little effect on the sensitivity of the cyclophosphamide-sensitive cells to this agent. More recently, Sladek and Landkamer [18] have confirmed and extended these findings by showing that several different inhibitors of aldehyde dehydrogenase activity were able to restore the sensitivity of oxazaphosphorine-resistant L1210 and P388 cells to oxazaphosphorines.

In the present investigation, known (disulfiram [31, 32], DDTC [32], cyanamide [33]) or suspected (EPP [18]) inhibitors of aldehyde dehydrogenase activity potentiated the cytotoxic action of ASTA Z 7557 and 4-hydroperoxycyclophosphamide toward CFU-S but not toward CFU-GM; they did not potentiate the cytotoxic action of acrolein or non-oxazaphosphorine cross-linking agents toward CFU-S or CFU-GM. These results suggest that CFU-S are relatively insensitive to oxazaphosphorines because they contain the aldehyde dehydrogenase isozyme(s) that catalyzes the oxidation of aldophosphamide to carboxyphosphamide, and that CFU-GM are relatively sensitive to oxazaphosphorines because they lack this enzyme activity. Several isozymes of aldehyde dehydrogenase have been found in various subcellular fractions of mammalian cells [34], but it is not known which isozyme(s) catalyzes the oxidation of aldophosphamide.

Since CFU-S are uncommitted hematopoietic stem cells capable of differentiation along the myeloid and other hematopoietic lines [35] whereas CFU-GM are progenitor cells committed to the myeloid line [36], the results suggest that there is a loss of aldehyde dehydrogenase activity upon differentiation. Whether or not this loss of activity occurs upon differentiation to other cell types, for example the erythroid and megakaryocytoid series, remains to be determined. In light of the fact that cyclophosphamide is relatively platelet sparing, loss of activity may not occur in the megakaryocytoid series. Investigations designed to quantify the sensitivity of murine erythroid progenitors (BFU-E and CFU-E) and megakaryocytoid progenitors (CFU-M) to oxazaphosphorines in the absence and presence of inhibitors of aldehyde dehydrogenase activity are currently in progress in our laboratory.

Consistent with our findings, Hilton and Colvin [17], upon examining several human leukemia cell lines, found a correlation between aldehyde dehydrogenase activity, sensitivity to activated cyclophosphamide, and the degree of differentiation. Cell lines believed to be most closely related to hematopoietic stem cells, e.g. HEL and K562, were relatively insensitive to activated cyclophosphamide and contained greater aldehyde dehydrogenase activity than did more committed cell lines, e.g. HL60 and

ML-1. Furthermore, disulfiram potentiated the cytotoxic action of activated cyclophosphamide toward the insensitive cell lines.

Our observations in mice and those of Hilton and Colvin [17] in human leukemia cells lines suggest that pluripotent hematopoietic stem cells, as compared to more committed hamatopoietic cells, may be relatively insensitive to oxazaphosphorines because of differentially greater aldehyde dehydrogenase activity. Evidence for a differential sensitivity to 4-hydroperoxycyclophosphamide between human bone marrow CFU-GM and more primitive hematopoietic cells has been presented by Beran and Zander [37]. They found that a concentration of 4-hydroperoxycyclophosphamide that eliminated CFU-GM present in human marrow cell suspensions when the marrow was initially obtained did not arrest the ability of these cell suspensions to generate CFU-GM following subsequent long-term Dexter culture. Moreover, Kaizer *et al.* [38], used 4-hydroperoxycyclophosphamide to purge residual tumor cells from marrow grafts prior to autologous transplantation in a phase I clinical trial and observed that a concentration of 4-hydroperoxycyclophosphamide that eliminated detectable CFU-GM from the graft marrow did not impair, with one exception, hematologic reconstitution. We are currently using the human CFU-GEMM (multipotent stem cell) and CFU-GM assays to more directly examine the above hypothesis.

Our finding that preincubation of bone marrow cell suspensions at 37° "sensitizes" CFU-S to oxazaphosphorines, while having little or no effect on the sensitivity of CFU-GM to these agents, has potential clinical significance since much effort has been focused recently on the use of oxazaphosphorines to purge residual tumor cells from autologous marrow grafts. If tumor cells behave similarly to CFU-GM, i.e. they are not "sensitized" by preincubation, then excessive handling time between the collection of donor marrow and drug exposure, and/or inappropriate handling during this time, may result in a decrease in the margin of safety necessary for a successful outcome.

The results of this investigation are supportive of the idea, first proposed over a decade ago [3–5, 14–16], that the relatively favorable therapeutic ratio exhibited by cyclophosphamide is due to favorable differences in the balance between the formation of active metabolites and conversion to inactive metabolites, as catalyzed by aldehyde dehydrogenases, in neoplastic and critical normal cells such as hematopoietic stem cells. Although it has been shown that resistance to oxazaphosphorines in neoplastic cells may result from an increase in aldehyde dehydrogenase activity [8, 18], it would not be advantageous to include an aldehyde dehydrogenase inhibitor in the treatment regimen to attempt to prevent or overcome this resistance if differences in aldehyde dehydrogenase activity are the basis for the favorable margin of safety in the first place. The observations that disulfiram potentiates cyclophosphamide-induced leukopenia [39] and host lethality [5, 40] without [5] or only slightly [39] increasing the antitumor action of cyclophosphamide support this conclusion.

REFERENCES

1. D. L. Hill, W. R. Laster, Jr. and R. F. Struck, *Cancer Res.* **32**, 658 (1972).
2. N. E. Sladek, *Cancer Res.* **33**, 651 (1973).
3. N. E. Sladek, *Cancer Res.* **33**, 1150 (1973).
4. P. J. Cox, B. J. Phillips and P. Thomas, *Cancer Res.* **35**, 3755 (1975).
5. P. J. Cox, B. J. Phillips and P. Thomas, *Cancer Treat. Rep.* **60**, 321 (1976).
6. B. E. Domeyer and N. E. Sladek, *Biochem. Pharmac.* **29**, 2903 (1980).
7. B. E. Domeyer and N. E. Sladek, *Biochem. Pharmac.* **30**, 2065 (1981).
8. J. Hilton and D. Cohen, *Proc. Am. Ass. Cancer Res.* **23**, 169 (1982).
9. H. W. Goedde, D. P. Agarwal and S. Harada, *Clin. Genet.* **16**, 29 (1979).
10. N. Brock and H-J. Hohorst, *Z. Krebsforsch.* **88**, 185 (1977).
11. O. M. Friedman, A. Myles and M. Colvin, *Adv. Cancer Chemother.* **1**, 143 (1979).
12. J. F. Powers and N. E. Sladek, *Cancer Res.* **43**, 1101 (1983).
13. N. E. Sladek, D. Doeden, J. F. Powers and W. Krivit, *Cancer Treat. Rep.* **68**, 1247 (1984).
14. B. E. Domeyer and N. E. Sladek, *Fedn Proc.* **33**, 581 (1974).
15. T. A. Connors, P. J. Cox, P. B. Farmer, A. B. Foster and M. Jarman, *Biochem. Pharmac.* **23**, 115 (1974).
16. P. J. Cox, P. B. Farmer and M. Jarman, *Biochem. Pharmac.* **24**, 599 (1975).
17. J. Hilton and M. Colvin, *Proc. Am. Ass. Cancer Res.* **25**, 339 (1984).
18. N. E. Sladek and G. J. Landkamer, *Cancer Res.* **45**, 1549 (1985).
19. W. Fried, A. Kedo and J. Barone, *Cancer Res.* **37**, 1205 (1977).
20. L. L. Sensenbrenner, J. J. Marini and M. Colvin, *J. natn. Cancer Inst.* **62**, 975 (1979).
21. J. E. Till and E. A. McCulloch, *Radiat. Res.* **14**, 213 (1961).
22. R. G. Worton, E. A. McCulloch and J. E. Till, *J. cell. Physiol.* **74**, 171 (1969).
23. R. J. Tallarida and R. B. Murray, *Manual of Pharmacologic Calculations*, p. 14. Springer-Verlag, New York (1981).
24. M. Stanulovic and S. Chaykin, *Archs Biochem. Biophys.* **145**, 27 (1971).
25. G. Voelcker, L. Bielicki and H-J. Hohorst, *J. Cancer Res. clin. Oncol.* **99**, A58 (1981).
26. H-J. Hohorst, L. Bielicki and G. Voelcker, in *Proceedings of the Thirteenth International Cancer Congress*, p. 384 (1982).
27. L. Bielicki, G. Voelcker and H. J. Hohorst, *J. Cancer Res. clin. Oncol.* **105**, 27 (1983).
28. J. E. Low, R. F. Borch and N. E. Sladek, *Cancer Res.* **43**, 830 (1982).
29. J. E. Low, R. F. Borch and N. E. Sladek, *Cancer Res.* **43**, 5815 (1983).
30. N. E. Sladek, J. F. Powers and G. M. Grage, *Drug Metab. Dispos.* **12**, 553 (1984).
31. D. I. Eneanya, J. R. Bianchine, D. O. Duran and B. D. Andresen, *A. Rev. Pharmac. Toxic.* **21**, 275 (1981).
32. R. A. Deitrich and V. G. Erwin, *Molec. Pharmac.* **7**, 301 (1971).
33. R. A. Deitrich, P. A. Troxell, W. S. Worth and V. G. Erwin, *Biochem. Pharmac.* **25**, 2733 (1976).
34. R. Pietruszko, in *Isozymes: Current Topics in Biological and Medical Research. Cellular Localization, Metabolism and Physiology* (Eds. M. C. Rattazzi, J. G. Scandalios and G. S. Whitt), Vol. 8, p. 195. Alan R. Liss, New York (1983).

35. D. Metcalf, *Haemopoietic Cells*, p. 74. North-Holland, Amsterdam (1971).
36. T. R. Bradley and D. Metcalf, *Aust. J. exp. Biol. med. Sci.* **44**, 287 (1966).
37. M. Beran and A. R. Zander, *Proc. Am. Ass. Cancer Res.* **25**, 377 (1984).
38. H. Kaizer, R. K. Stuart, D. J. Fuller, H. G. Braine, R. Saral, M. Colvin, M. D. Wharam and G. W. Santos, *Proc. Am. Soc. clin. Oncol.* **1**, 131 (1982).
39. M. P. Hacker, W. B. Ershler, R. A. Newman and R. L. Gamelli, *Cancer Res.* **42**, 4490 (1982).
40. H. Habs, M. Habs and D. Schmähl, *Arzneimittel-Forsch.* **31**, 530 (1981).