Potentiation of the Cytotoxic Action of Mafosfamide by $N$-Isopropyl-$p$-formylbenzamide, a Metabolite of Procarbazine

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ABSTRACT

Several mouse aldehyde dehydrogenases catalyze the detoxification of aldophosphamide, the pivotal metabolite of the prodrugs cyclophosphamide, mafosfamide, and other oxazaphosphorines. $N$-Isopropyl-$p$-formylbenzamide, a major metabolite of procarbazine, was found to be an excellent substrate ($K_m = 0.84 \mu M$) for at least one of these enzymes, namely, mouse aldehyde dehydrogenase-2. The $K_m$ for mouse aldehyde dehydrogenase-2-catalyzed detoxification of aldophosphamide is 16 $\mu M$. Thus, competition between $N$-isopropyl-$p$-formylbenzamide and aldophosphamide for the catalytic site on the enzyme should strongly favor the former, and the rate at which aldophosphamide is detoxified should be markedly retarded. Mouse L1210/OAP and P388/CLA leukemia cells are relatively insensitive to the oxazaphosphorines because they contain large amounts of mouse aldehyde dehydrogenase-2. As predicted, $N$-isopropyl-$p$-formylbenzamide markedly potentiated the cytotoxic action of mafosfamide against these cells. Mouse L1210/O and P388/O lack the enzyme. Again as expected, $N$-isopropyl-$p$-formylbenzamide essentially did not potentiate the cytotoxic action of mafosfamide against these cells.

Certain mouse and human hematopoietic progenitor cells also contain an aldehyde dehydrogenase that catalyzes the detoxification of aldophosphamide, but the specific identity of this enzyme remains to be established. $N$-Isopropyl-$p$-formylbenzamide potentiated the cytotoxic action of mafosfamide against these cells as well. Clinically, procarbazine and the oxazaphosphorines are used to treat certain neoplastic diseases. Frequently, they are used in combination. Our findings demonstrate the potential for both desirable and undesirable drug interactions when these agents are used concurrently. Similar drug interactions can be expected when other substrates for, or inhibitors of, the relevant aldehyde dehydrogenase(s) are used. Mouse aldehyde dehydrogenase-2. The $K_m$, for mouse aldehyde dehydrogenase-2, has been identified as AHD-2 (10, 14, 15, 19-22). The aldehyde dehydrogenase expressed in L1210/OAP has been identified as AHD-2. The aldehyde dehydrogenase expression in L1210/OAP has been identified as AHD-2. The aldehyde dehydrogenase expression in L1210/OAP has been identified as AHD-2.

INTRODUCTION

Cyclophosphamide and procarbazine are antineoplastic agents that are frequently included in combination chemotherapy regimens, e.g., COPP (cyclophosphamide, vincristine, prednisone, procarbazine) (1); CAMP (cyclophosphamide, Adriamycin, methotrexate, procarbazine) (2); PROVECIP (procarbazine, vinblastine, cyclophosphamide, prednisone) (3, 4); and CABOPP (cyclophosphamide, doxorubicin, bleomycin, vincristine, procarbazine, prednisone) (5, 6). The combination of cyclophosphamide and procarbazine may also have some utility in the treatment of cancer in large part due to the favorable margin of safety exhibited by this agent. The latter process is catalyzed by NAD(P)-linked aldehyde dehydrogenases.

Greater expression of the relevant aldehyde dehydrogenase(s) in critical normal tissues versus that in tumor tissue has been suggested as the basis for the favorable margin of safety exhibited by cyclophosphamide and other oxazaphosphorine nitrogen mustards, e.g., mafosfamide (reviewed in Ref. 8). Expression of the relevant aldehyde dehydrogenase activity has been demonstrated in murine and human multipotent and committed hematopoietic progenitor cells (9-13), and in murine intestinal crypt and villus cells (14), but not in a number of murine and human tumor cell lines (10, 15). These differences in expression of the relevant aldehyde dehydrogenase activity can, at least partially, explain the relative differences in sensitivity of these cells to the oxazaphosphorines.

Aldehyde dehydrogenases, each exhibiting unique characteristics, e.g., substrate preferences/specificity, tissue distribution, and sensitivity to inhibitors, have been identified in a variety of animal species (16, 17). Ten different aldehyde dehydrogenases that are capable of catalyzing the detoxification of aldophosphamide have been identified in mouse tissues (18). Nine are found in the liver. One, namely, AHD-2, accounts for the bulk of the total hepatic activity when the aldophosphamide concentration is pharmacological (17, 18). It is found in the cytosol. This is not the hepatic aldehyde dehydrogenase that is most important in catalyzing the oxidation of acetaldehyde. That distinction goes to a mitochondrial enzyme, AHD-5 (17, 18).

Expression of a relevant aldehyde dehydrogenase has also been implicated as a mechanism of acquired resistance to the oxazaphosphorines. Certain murine tumor cell lines, e.g., L1210 and P388, that ordinarily lack the relevant aldehyde dehydrogenase activity and are sensitive to the oxazaphosphorines, become resistant to them when repeatedly exposed to increasing amounts of these agents. In at least some cases, e.g., L1210/OAP and P388/CLA, cells made resistant by this treatment exhibit the relevant aldehyde dehydrogenase(s) (10, 14, 15, 19-22). The aldehyde dehydrogenase expressed in L1210/OAP has been identified as AHD-2.

Like cyclophosphamide, procarbazine is a prodrug. The parent compound is oxidized to the primary circulating metabolite, azoprocabazine, by the hepatic microsomal P-450 system and by monoamine oxidase. Azoprocabazine can be further metabolized (reviewed in Ref. 8). Cyclophosphamide is a prodrug. It is converted to the primary circulating metabolite, 4-hydroxycyclophosphamide, which exists in equilibrium with its ring-opened tautomer aldophosphamide; neither of these, per se, is toxic to cells. Aldophosphamide undergoes one of two fates: $\beta$ elimination to phosphoramid mustard, the ultimate toxic species, or oxidation to carboxyphosphamide, a nontoxic product. The latter process is catalyzed by NAD(P)-linked aldehyde dehydrogenase(s).
olized along any of four pathways (23–26). Of the four, two
give rise to the aldehyde metabolite, N-isopropyl-p-formylben-
zamide. This compound is rapidly converted to N-isopropylter-
ephthalamic acid by aldehyde dehydrogenase(s) (26, 27).

In view of the foregoing, the potential for a significant cyclo-
phosphamide-procarbazine drug interaction is obvious. Thus,
the purpose of the present investigation was to test the notion
that N-isopropyl-p-formylbenzamide, acting as an alternative
substrate, inhibits aldehyde dehydrogenase-catalyzed detoxifi-
cation of cyclophosphamide, thereby sensitizing cells wherein
this reaction ordinarily occurs, to this agent.

MATERIALS AND METHODS

Azoprocabazine, azoxy-I-procarbazine, and N-isopropyl-p-formyl-
benzamide were provided by Dr. Russell A. Prough (University of
Louisville, Louisville, KY). Mafosfamide (2-bis-(2-chloroethyl)-ammo]-
4-(2-sulfoethylthio)-tetrahydro-2//-1,3,2-oxazaphosphorine-2-oxide-
cyclohexylamine was provided by Dr. P. Hilgard (Asta-Werke AG,
Bielefeld, Federal Republic of Germany). Procarbazine was obtained
from Mr. Frank F. Sorter (Hoffmann-La Roche Inc., Nutley, NJ).
Phosphoramid mustard-cyclohexylamine and conditioned medium
from phytohemagglutinin-stimulated human peripheral blood leuko-
cytes were supplied by Mr. L. H. Kedda (Drug Synthesis and Chemistry
Branch, Division of Cancer Treatment, National Cancer Institute,
Bethesda, MD), and Dr. F. Uckun (Department of Therapeutic Rادي,
University of Minnesota, Minneapolis, MN), respectively. NAD* was purchased from Sigma Chemical Co., St. Louis, MO.

Procarbazine, azoprocabazine, azoxy-I-procarbazine, and N-isopro-
pyl-p-formylbenzamide were dissolved in 95% ethanol. All other drugs
were dissolved in triple-distilled water or drug-exposure media and
sterilized by passage through 0.22-μm Millipore filters. Drugs were
used within 1 h of preparation and were kept on ice until use. Drug-
exposure medium was a phosphate-buffered, saline-based solution, pH
7.4, prepared as previously described (20).

CFU-S experiments. These animals were obtained from Taconic, Ger-
town, NY. Animals were housed in plastic cages fitted with filtered
lids and were given standard laboratory food and water ad libitum. A
strict 12-h photoperiod was maintained.

Cultured mouse LI210 and P388 cells, sensitive (LI210/0, P388/0)
cells to drugs. /V-Isopropyl-p-formylbenzamide or vehicle was added
exactly 3 min prior to the addition of cytotoxic agent or vehicle, after
which the cells were incubated at 37°C for 30 min.

The sensitivity of human hematopoietic stem cells to mafosfamide
with or without N-isopropyl-p-formylbenzamide was determined as
previously described (10). Human bone marrow cells were obtained
from a healthy adult volunteer and were prepared for assay as previously
described (10).

Aldehyde dehydrogenase-catalyzed oxidation of acetaldehyde and N-
isopropyl-p-formylbenzamide was quantified as described by Manthey
et al. (18, 28). Km values for the catalysis of N-isopropyl-p-formyl-
benzamide by AHD-2 were determined by the integrated Michaelis method
of analysis of single enzyme progress curves (31).

RESULTS

Preliminary experiments revealed that N-isopropyl-p-formyl-
benzamide, but not procarbazine itself, inhibited the oxidation of
acetaldehyde to acetic acid catalyzed by a crude soluble fraction
obtained from mouse liver, and that it was a substrate for the
aldehyde dehydrogenase(s) present therein (data not presented).

N-Isopropyl-p-formylbenzamide was an excellent substrate
for AHD-2; the Km value was 0.84 μM, Fig. 1. The Km value
defining the metabolism of an agent, e.g., N-isopropyl-p-formyl-
benzamide, equals the K, value defining competitive inhi-
bition by this agent of the metabolism of a second agent, e.g.,
adophosphamide, when the two are alternative substrates for
the same enzyme (33). Given the preceding and the fact that
the Km value for AHD-2-catalyzed oxidation of aldophospham-
ide is 16 μM (18), N-isopropyl-p-formylbenzamide should mark-

![Fig. 1. N-isopropyl-p-formylbenzamide oxidation catalyzed by AHD-2: inte-
grated Michaelis equation kinetic analysis. A complete enzyme progress curve of
the total consumption of initially saturating concentrations (15 μM) of N-isopro-
pyl-p-formylbenzamide by AHD-2 was generated and analyzed as described in
"Materials and Methods." Points represent a single analysis; the line was drawn by
least squares regression analysis from which the Km (negative inverse of the
slope) was determined. This experiment was repeated 2 more times. The mean
Km value for the 3 determinations was 0.84 ± 0.37 μM.](chart.png)
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**Fig. 2. Sensitivity of cultured murine tumor cells to mafosfamide and phosphoramide mustard in the presence of N-isopropyl-p-formylbenzamide.** Vehicle (O) or N-isopropyl-p-formylbenzamide was added to tumor cell suspensions. Exactly 3 min later, vehicle (C), mafosfamide (●), or phosphoramide mustard (□) was added, and the cells were incubated for 30 min at 37°C. Concentrations of cytotoxic agents were 21 (L1210/0), 155 (L1210/OAP), 5 (P388/0), and 35 (P388/CLA) μM mafosfamide, and 114 (L1210/0), 110 (L1210/OAP), 50 (P388/0), and 100 (P388/CLA) μM phosphoramide mustard. Cells were harvested at the end of the incubation period and were subsequently resuspended and grown in drug-free medium. Surviving fractions were determined by the back-extrapolation method as described in "Materials and Methods." Points represent the mean of measurements on duplicate cultures.

**Fig. 3. Sensitivity of murine L1210/OAP cells to mafosfamide or phosphoramide mustard in the presence of N-isopropyl-p-formylbenzamide.** Vehicle (C) or 200 μM N-isopropyl-p-formylbenzamide (●) was added to tumor cell suspensions. Exactly 3 min later, vehicle or cytotoxic agent was added and cells were incubated at 37°C for 30 min. Cells were harvested at the end of the incubation period and were subsequently resuspended and grown in drug-free medium. Surviving fractions were determined by the back-extrapolation assay as described in "Materials and Methods." Points represent the mean of measurements on duplicate cultures. The responses of cultured L1210/0 cells to mafosfamide are included for comparative purposes (□).

**Fig. 4. Sensitivity of murine P388/CLA cells to mafosfamide or phosphoramide mustard in the presence of N-isopropyl-p-formylbenzamide.** Vehicle (C) or 200 μM N-isopropyl-p-formylbenzamide (●) was added to tumor cell suspensions. Exactly 3 min later, vehicle or cytotoxic agent was added and cells were incubated at 37°C for 30 min. Cells were harvested at the end of the incubation period and were subsequently resuspended and grown in drug-free medium. Surviving fractions were determined by the back-extrapolation assay as described in "Materials and Methods." Points represent the mean of measurements on duplicate cultures. The response of cultured P388/0 cells to mafosfamide are included for comparative purposes (□).

Collectively, these observations are consistent with the finding that N-isopropyl-p-formylbenzamide is an excellent substrate for AHD-2, and the notion that it competitively inhibits AHD-2-catalyzed detoxification of aldoephosphamide.

In order to quantify the effect of N-isopropyl-p-formylbenzamide on the cytotoxic action of mafosfamide and phosphoramide mustard, L1210/OAP and P388/CLA cells were exposed to increasing concentrations of mafosfamide or phosphoramide mustard in the presence of 200 μM N-isopropyl-p-formylbenzamide; Figs. 3 and 4. LC₉₀ values for mafosfamide and phosphoramide mustard in the presence and absence of N-isopropyl-p-formylbenzamide were calculated from these data. The presence of N-isopropyl-p-formylbenzamide reduced the LC₉₀ values for mafosfamide from 176 to 17 μM (L1210/OAP), and from 44 to 6 μM (P388/CLA). These observations reflect essentially full restoration of sensitivity to mafosfamide by N-isopropyl-p-formylbenzamide, since LC₉₀ values for mafosfamide against wild type L1210/0 and P388/0 cells are 14 and 7 μM, respectively.

The presence of N-isopropyl-p-formylbenzamide also reduced the rate at which AHD-2 catalyzes the oxidation of aldoephosphamide when both agents are present.

Effect of N-Isopropyl-p-formylbenzamide on the Cytotoxic Action of Mafosfamide against Murine Tumor Cell Lines. Preliminary experiments established that 30-min exposure to procarbazine (1 mM), or any of three of its metabolites, namely, azoprocabarbazine (0.3 mM), azyoxy-1-procarbazine (0.3 mM), and N-isopropyl-p-formylbenzamide (1 mM), was not toxic to cultured L1210 cells (data not presented). Moreover, azoprocabarbazine (0.3 mM) and azyoxy-1-procarbazine (0.3 mM) did not potentiate the cytotoxic action of mafosfamide, an oxazaphosphorine nitrogen mustard which spontaneously gives rise to 4-hydroxycyclophosphamide in physiological solutions, against cultured oxazaphosphorine-resistant L1210/OAP cells (data not presented). However, a marked, dose-dependent potentiation of the cytotoxic activity of mafosfamide against L1210/OAP, as well as against oxazaphosphorine-resistant P388/CLA, cells, was observed when N-isopropyl-p-formylbenzamide was included in the incubation media; Fig. 2. Like L1210/OAP cells, P388/CLA cells contain an aldehyde dehydrogenase that catalyzes the detoxification of aldoephosphamide (14, 20, 21). In contrast, potentiation by N-isopropyl-p-formylbenzamide of the cytotoxic activity of mafosfamide against the oxazaphosphorine-sensitive L1210/0 and P388/0 cells was marginal at best. These cells lack aldehyde dehydrogenases capable of catalyzing the oxidation of aldoephosphamide (10, 19–21). A relatively small amount of potentiation of the cytotoxic action of phosphoramide mustard, a cyclophosphamide metabolite not susceptible to oxidation catalyzed by aldehyde dehydrogenases, was also observed. Collectively, these observations are consistent with the finding that N-isopropyl-p-formylbenzamide is an excellent substrate for AHD-2, and the notion that it competitively inhibits AHD-2-catalyzed detoxification of aldoephosphamide.
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Fig. 5. Sensitivity of murine CFU-S, CFU-GEMM, CFU-Mk, and CFU-GM to a fixed concentration of mafosfamide or phosphoramide mustard in the presence of increasing concentrations of N-isopropyl-p-formylbenzamide. Vehicle or N-isopropyl-p-formylbenzamide was added to BALB/c bone marrow cell suspensions. Exactly 3 min later, vehicle (O), mafosfamide (•), or phosphoramide mustard (D) was added, and cells were incubated for 30 min at 37°C. Cells were harvested at the end of the incubation period, resuspended in drug-free medium, and assayed for CFU-S, CFU-GEMM, CFU-Mk, and CFU-GM as described in “Materials and Methods.” Concentrations of mafosfamide were 40 (CFU-S), 50 (CFU-GEMM), 35 (CFU-Mk), and 20 (CFU-GM) µM. The concentration of phosphoramide mustard was 64 µM (CFU-S). Points are mean ± SE of observations made in 3–4 mice (day 12, CFU-S), or mean ± SE of triplicate plates (CFU-GEMM, CFU-Mk, CFU-GM). Results are expressed as percentage of control (exposed to vehicle only). *, significantly different from control, P ≤ 0.05; **, significantly different from mafosfamide, P ≤ 0.05.

Fig. 6. Sensitivity of murine CFU-GEMM to mafosfamide in the absence and presence of a fixed concentration of N-isopropyl-p-formylbenzamide. Vehicle (C) or 10 µM N-isopropyl-p-formylbenzamide (•) was added to BALB/c bone marrow cell suspensions. Exactly 3 min later, vehicle or mafosfamide was added, and cells were incubated for 30 min at 37°C. Following incubation, cells were harvested, resuspended in drug-free medium, and assayed for CFU-GEMM as described in “Materials and Methods.” N-Isopropyl-p-formylbenzamide alone was without cytotoxic activity. Results are expressed as percentage of inhibition of the relevant control. Points are the mean of three experiments; bars, SE.

The effect of N-isopropyl-p-formylbenzamide on the toxic action of mafosfamide against hematopoietic progenitor cells was examined to determine if N-isopropyl-p-formylbenzamide would potentiate the toxic action of the oxazaphosphorines against critical normal tissues known to express an aldehyde dehydrogenase that catalyzes the detoxification of aldoephosphamide, e.g., mouse CFU-S, CFU-GEMM, and CFU-Mk, but not mouse CFU-GM (9, 11, 12).

N-isopropyl-p-formylbenzamide itself was not toxic to mouse CFU-S, CFU-GEMM, or CFU-Mk, and only marginally toxic to mouse CFU-GM; Fig. 5. It potentiated the cytotoxic action of mafosfamide against CFU-S, CFU-GEMM, and CFU-Mk; it did not potentiate the cytotoxic action of mafosfamide against CFU-GM, nor did it potentiate the cytotoxic action of phosphoramide mustard against CFU-S. These observations are in keeping with the notions that (a) mouse L1210/OAP and P388/CLA leukemia cells, mouse CFU-S, CFU-GEMM, and CFU-Mk are capable of detoxifying cyclophosphamide because they contain an enzyme, namely, aldehyde dehydrogenase, that catalyzes such a detoxification; (b) N-isopropyl-p-formylbenzamide, because it too is a substrate for the enzyme, slows the rate at which detoxification occurs; and (c) CFU-GM lacks the relevant aldehyde dehydrogenase.

To quantify the potentiation observed in the preceding experiments, CFU-GEMM's present in mouse bone marrow were exposed to increasing concentrations of mafosfamide and a fixed concentration (10 µM) of N-isopropyl-p-formylbenzamide, and LC50 values were determined; Fig. 6. This value was 56 and 10 µM when N-isopropyl-p-formylbenzamide was omitted from and included in, respectively, the exposure media.

Human hematopoietic progenitor cells, e.g., CFU-Mix, CFU-Mk, CFU-GM, and BFU-E, also express aldehyde dehydrogenase(s) capable of catalyzing the detoxification of aldoephosphamide (10). N-isopropyl-p-formylbenzamide potentiated the cytotoxic action of mafosfamide against these cells as well; Fig. 7.

Fig. 7. Sensitivity of human progenitor cells to a fixed concentration of mafosfamide in the presence of increasing concentrations of N-isopropyl-p-formylbenzamide. Vehicle or N-isopropyl-p-formylbenzamide was added to mononuclear bone marrow cell suspensions. Exactly 3 min later, vehicle (C) or 20 µM mafosfamide (•) was added and cells were incubated for 30 min at 37°C. Cells were harvested at the end of the incubation period, resuspended in drug-free medium, and assayed for CFU-Mix, CFU-Mk, CFU-GM, and BFU-E. Control colony numbers were 24 (CFU-Mix), 40 (CFU-Mk), 200 (CFU-GM), and 175 (BFU-E). Points, mean of measurements made on duplicate plates; bars, range. Results are expressed as percentage of control (exposed to vehicle only).
DISCUSSION

The results of the present investigation demonstrate that a metabolite of procarbazine, namely, N-isopropyl-p-formylbenzamide, is an excellent substrate for mouse AHD-2 (Km = 0.84 μM). The expectation is that, as an alternative substrate, N-isopropyl-p-formylbenzamide would be a good competitive inhibitor of aldophosphamide oxidation catalyzed by this enzyme (Km = 16 μM). Manthey et al., (18) demonstrated that this enzyme accounts for the bulk of total hepatic aldehyde dehydrogenase-catalyzed oxidation of aldophosphamide. Taken together, these observations suggest that N-isopropyl-p-formylbenzamide might reduce hepatic clearance of aldophosphamide when procarbazine and cyclophosphamide are administered concurrently. However, because the liver contains such a large excess of AHD-2, hepatic clearance of aldophosphamide is thought to be a flow-limited process (28), and it is therefore unlikely that inhibition of hepatic AHD-2 by N-isopropyl-p-formylbenzamide would potentiate the systemic action of cyclophosphamide. Further complicating this prognostication is that several other aldehyde dehydrogenases capable of catalyzing the oxidation of aldophosphamide have been identified in mouse liver (18). It is not known whether N-isopropyl-p-formylbenzamide is a substrate for these enzymes, although that would seem likely.

The foregoing does not, however, preclude the possibility that local inhibition of a relevant aldehyde dehydrogenase by N-isopropyl-p-formylbenzamide in critical normal tissues which may express smaller amounts of this activity, e.g., bone marrow hematopoietic progenitor cells, might enhance the toxicity of mafosfamide against such tissues/organs. The results of the present investigation suggest that this is, in fact, a possible outcome of combined administration of procarbazine and cyclophosphamide. We observed a sizeable potentiation of the toxicity of mafosfamide against both murine and human hematopoietic progenitor cells at a very low concentration (10 μM) of N-isopropyl-p-formylbenzamide.

Potentiation of cyclophosphamide-induced bone marrow toxicity by procarbazine in vivo would be expected only if sufficiently high concentrations of N-isopropyl-p-formylbenzamide were generated. This would seem likely given that (a) 1 h after the p.o. administration of procarbazine to rats, 72% of total drug found in plasma was N-isopropyl-p-formylbenzamide (25); (b) the usual human dose of procarbazine is 100 to 200 mg/m²/day (34); (c) up to 70% of the administered human dose is recovered in the urine as N-isopropylterephthalamic acid within 24 h of p.o. or parenteral procarbazine administration (34); (d) N-isopropylterephthalamic acid is the metabolite generated when N-isopropyl-p-formylbenzamide is oxidized (26); and (e) in our models, substantial potentiation was observed at concentrations of N-isopropyl-p-formylbenzamide as low as 10 μM. Moreover, procarbazine is known to produce a "disulfiram-like" effect, namely, nausea, vasodilation, headache, etc., when alcohol is ingested following its administration (35). This reaction is attributed to the accumulation of acetaldehyde resulting from the inhibition of aldehyde dehydrogenase(s). In the case of procarbazine, inhibition could be by N-isopropyl-p-formylbenzamide, meaning that the concentration of this metabolite is sufficiently high to do so. Alternatively, decreased enzyme activity could be due to irreversible inhibition of the enzyme or a reduction in the amount of enzyme present affected by procarbazine or one of its metabolites. Thus, aldehyde dehydrogenase-catalyzed oxidation of acetaldehyde by a non-aldehydized hepatic-soluble fraction was reduced to 37% of control values when livers were obtained from female DBA/2 mice that had repeatedly been given a relatively high dose of procarbazine, namely, 100 mg/kg, i.p., 96, 72, 48, 24, and 1 h before sacrifice (data not presented). Vasiliiou et al. (36) have made similar observations in rats.

We know of no clear clinical example of increased toxicity due to the anticipated drug interaction but, perhaps relevant, the in vivo toxic action of cyclophosphamide on mouse intestinal crypt cells was markedly increased when an inhibitor of aldehyde dehydrogenase, namely, 4-(diethylamino)-benzaldehyde, was coadministered with cyclophosphamide; 4-(diethylamino)-benzaldehyde and N-isopropyl-p-formylbenzamide are structurally quite similar (14, 37). It should also be noted that the postulated drug interaction may be advantageous when the therapeutic objective is to prevent the graft rejection that otherwise often occurs after bone marrow transplantation; indeed, there is some evidence to support this notion (7).

N-isopropyl-p-formylbenzamide also potentiated the cytotoxic action of mafosfamide against oxazaphosphorine-resistant mouse L1210/OAP and P388/CLA tumor cells. These cell lines are resistant to oxazaphosphorines because they contain relatively high levels of an aldehyde dehydrogenase that catalyzes the detoxification of aldophosphamide. High levels of a relevant aldehyde dehydrogenase are likely to be found in human tumor populations that become relatively insensitive to the oxazaphosphorines (but not to other antitumor agents) after repeated exposure to them, although there is currently no evidence that this occurs clinically. However, it has been demonstrated that human mammary and melanoma cell lines cultured in media containing progressively increasing concentrations of an oxazaphosphorine become resistant only to the oxazaphosphorines (38, 39), suggesting that the underlying biological basis for the oxazaphosphorine-specific resistance is an increase in a relevant aldehyde dehydrogenase. Measurable amounts of relevant aldehyde dehydrogenase activity were present in 4 Wilms tumors and a testicular tumor. Colvin and Hilton (40) detected aldehyde dehydrogenase activity in several human tumor cell lines, but it is not clear whether the activity was relevant, since the substrate used for these studies was not identified. Potentially, procarbazine (N-isopropyl-p-formylbenzamide) could sensitize neoplastic cells that contain the relevant aldehyde dehydrogenase activity to cyclophosphamide and other oxazaphosphorines, thus providing an acceptable rationale for combining these two agents when treating such neoplasias. The downside is that, as discussed above, certain critical normal cells, e.g., bone marrow hematopoietic cells, would also become more sensitive to the oxazaphosphorine, so that there may be no net improvement in the therapeutic index (margin of safety) of the oxazaphosphorine, and when the tumor cells lacked the relevant aldehyde dehydrogenase activity, the margin of safety would actually decrease. The relevant aldehyde dehydrogenase activity was not present in 87 human primary leukemias and lymphomas obtained from oxazaphosphorine-naive patients.

The significance of the present investigations extends beyond potential drug interactions between procarbazine and cyclophosphamide. Review of the literature reveals >30 drugs which are reported to produce a disulfiram-like effect. Many of these, e.g., chloramphenicol, chloral hydrate, and methyltetrazole-thiol-containing cephalosporins, are used as adjunctive therapy

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* C. L. Manthey and N. E. Sladek, unpublished observations.
* N. E. Sladek and F. M. Uckun, unpublished observations.
in the treatment of cancer patients receiving cyclophosphamide. Obvious are the clinical consequences if, like V-isopropyl-p-formylbenzamide, these agents inhibit the human aldehyde dehydrogenase(s) that contribute to the detoxification of aldo-

REFERENCES


3. Host, H., and Abrahamsen, A. F. Combination chemotherapy with cyclo-

4. Host, H., and Abrahamsen, A. F. Combination chemotherapy with cyclo-

5. Host, H., and Abrahamsen, A. F. Combination chemotherapy with cyclo-


14. Ruso, J. E., Hilton, J., and Colvin, O. M. The role of aldehyde dehydro-

15. Manthey, C. L. Resolution and characterization of the aldehyde dehydrogen-


20. Sladek, N. E., Low, J. E., and Landkamer, G. J. Collateral sensitivity to cross-linking agents exhibited by cultured L1210 cells resistant to oxaza-


22. Ruso, J. E., and Hilton, J. Characterization of cytosolic aldehyde dehydro-


27. Oliverio, V. T., Denham, C., DeVita, V. T., and Kelly, M. G. Some phar-

28. Sladek, N. E. Metabolism of oxazaphosphorines. Pharmacol. Ther., 37: 301-


38. Hotel, J., Hauquitz, D., and Hilton, J. Inhibition of mouse cytosolic aldehyde dehydrogenase by (4-dihethylamino)benzaldehyde. Biochem. Phar-


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