Pergamon

Biochemical Pharmacology, Vol. 49, No. 5, pp. 669–675, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00503-6

PHENOLIC ANTIOXIDANT-INDUCED OVEREXPRESSION OF CLASS-3 ALDEHYDE DEHYDROGENASE AND OXAZAPHOSPHORINE-SPECIFIC RESISTANCE

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(*Received* 23 *June* 1994; *accepted* 3 *October* 1994)

Abstract—High-level cytosolic class-3 aldehyde dehydrogenase (ALDH-3)-mediated oxazaphosphorine-specific resistance (>35-fold as judged by the concentrations of mafosfamide required to effect a 90% cell-kill) was induced in cultured human breast adenocarcinoma MCF-7/0 cells by growing them in the presence of 30 μM catechol for 5 days. Resistance was transient in that cellular sensitivity to mafosfamide was fully restored after only a few days when the inducing agent was removed from the culture medium. The operative enzyme was identified as a type-1 ALDH-3. Cellular levels of glutathione *S*-transferase and DT-diaphorase activities, but not of cytochrome P450 IA1 activity, were also elevated. Other phenolic antioxidants, e.g. hydroquinone and 2,6-di-*tert*-butyl-4-hydroxytoluene, also induced ALDH-3 activity when MCF-7/0 cells were cultured in their presence. Thus, the increased expression of a type-1 ALDH-3 and the other enzymes induced by these agents was most probably the result of transcriptional activation of the relevant genes via antioxidant responsive elements present in their 5′-flanking regions. Cellular levels of ALDH-3 activity were also increased when a number of other human tumor cell lines, e.g. breast adenocarcinoma MDA-MB-231, breast carcinoma T-47D and colon carcinoma HCT 116b, were cultured in the presence of catechol. These findings should be viewed as greatly expanding the number of recognized environmental and dietary agents that can potentially negatively influence the sensitivity of tumor cells to cyclophosphamide and other oxazaphosphorines.

Key words: aldehyde dehydrogenase; phenolic antioxidants; antioxidant responsive elements; cyclophosphamide; aldophosphamide; mafosfamide; oxazaphosphorines; breast cancer; drug resistance

Phenolic antioxidants, e.g. catechol, BHA† and BHT, are known to induce glutathione *S*-transferase and DT-diaphorase (NAD(P)H:quinone oxidoreductase) activities in various human and rodent organs/tissues/cells [reviewed in Refs. 1 and 2]. Transcriptional activation of the relevant genes by these agents appears to be via ARE present in the 5′-flanking regions of these genes [3–6]. The ARE core sequence required for transcriptional activation of rat glutathione *S*-transferase Ya subunit and DT-diaphorase genes is reportedly 5′-GTGACNNNGC-3′ [4]. This sequence is also found in the 5′-flanking region (−738 to −729) of the rat ALDH-3 gene [7] as well as in that (−462 to −453) of the human DT-diaphorase gene [8, 9]. On the basis of the foregoing, and because cytosolic ALDH-3 mRNA levels are elevated significantly in mouse Hepa 1 cells cultured in the presence of *tert*-butylhydroquinone [10], we reasoned that, since a functional ARE was likely to also be present in the 5′-flanking region of the human ALDH-3 gene, phenolic antioxidants may induce ALDH-3 activity in human organs/tissues/cells, e.g. breast adenocarcinoma MCF-7/0 cells, and, since cellular sensitivity to oxazaphosphorines such as 4-hydroperoxycyclophosphamide and mafosfamide decreases as cellular content of ALDH-3 increases [11–14], phenolic antioxidant-treated cells, e.g. MCF-7/0, may be less sensitive to the oxazaphosphorines than are their untreated counterparts. These expectations were dramatically realized.

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† Abbreviations: BHA, 3(5)-di-*tert*-butyl-4-hydroxyanisole; BHT, 2,6-di-*tert*-butyl-4-hydroxytoluene; ARE, antioxidant responsive elements; ALDH-3, cytosolic class-3 aldehyde dehydrogenase; XRE, xenobiotic responsive elements; mIU, milli-International Unit of enzyme activity (nmol NAD(P)H formed/min in the case of aldehyde dehydrogenase activity, nmol of the conjugate of 1-chloro-2,4-dinitrobenzene and glutathione formed/min in the case glutathione *S*-transferase activity, nmol of 2,6-dichlorophenol-indophenol reduced/min in the case of DT-diaphorase activity, and nmol of resorufin formed/min in the case of cytochrome P450 IA1 activity); and LC_{90}, drug concentration required to effect a 90% cell-kill.

MATERIALS AND METHODS

Mafosfamide and phosphoramide mustard-cyclohexylamine were supplied by Dr. J. Pöhl, Asta-Werke AG, Bielefeld, Germany, and the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, respectively. [γ-^{32}P] ATP and [α-^{32}P] dATP were purchased from New England Nuclear Research Products-Du Pont Co., Boston, MA. T4 polynucleotide kinase

and a random primed DNA labeling kit were purchased from the USB Corp., Cleveland, OH. An oligonucleotide probe, viz. 5′-ATCCAGCAGCT GGAGGCGCTGCAGCGC-3′, specific (100% homology with cDNA [15]) for the mRNA sequence that codes for the 9 N-terminal end amino acids of stomach mucosa ALDH-3, was synthesized for us by the Microchemical Facility, University of Minnesota Medical School, Minneapolis, MN. 5′-End-labeling of this probe was with T4 polynucleotide kinase and [γ-^{32}P] ATP (3000 Ci/mmol) according to the protocol provided by the USB Corp. Full-length β-actin cDNA was provided by Dr. C. Campbell, Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN. Random primed labeling of β-actin cDNA was with the random primed labeling kit and [α-^{32}P] dATP (3000 Ci/mmol) according to the protocol provided by the USB Corp. All other chemicals, reagents and supplies were purchased from commercial sources or prepared as before [11, 13].

Purified stomach mucosa type-1 ALDH-3 and MCF-7/OAP type-2 ALDH-3, and anti-stomach mucosa type-1 ALDH-3 IgY, were prepared as described previously [11].

Human breast adenocarcinoma MCF-7/0 and MCF-7/OAP cells were originally obtained from Dr. B. Teicher, Dana-Farber Cancer Institute, Boston, MA. Human breast adenocarcinoma SK-BR-3 cells were provided by Dr. S. Ramakrishnan, Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN. Human breast adenocarcinoma MDA-MB-231, and human breast carcinoma T-47D and ZR-75-1, cells were purchased from the American Type Culture Collection, Rockville, MD. Human colon carcinoma cells, viz. colon C and HCT 116b, were provided by Dr. M. G. Brattain, Department of Biochemistry and Molecular Biology, Medical School of Ohio, Toledo, OH.

T-47D and ZR-75-1, and colon C and HCT 116b, cells were cultured (monolayer) in, respectively, RPMI-1640 medium/10% fetal bovine serum and Dulbecco's modified Eagle's medium/10% horse serum, each of which was supplemented with L-glutamine (2 mM), sodium bicarbonate (3.7 g/L), basal medium Eagle amino acids solution (6 mL/L), basal medium Eagle vitamin solution (6 mL/L), sodium pyruvate (1 mM), and gentamicin (50 mg/L). Population doubling times were 48, 88, 25 and 22 hr for T-47D, ZR-75-1, colon C and HCT 116b cells, respectively. All other cell lines were cultured at 37° as described previously [11, 13].

Catechol, hydroquinone, *tert*-butylhydroquinone, BHA, BHT, ethoxyquin and Vitamin E were dissolved in dimethyl sulfoxide. The concentration of dimethyl sulfoxide ultimately present in the culture media never exceeded 0.1% and did not affect cell growth or induce aldehyde dehydrogenase, glutathione *S*-transferase, DT-diaphorase or cytochrome P450 IA1 activities.

Lubrol-treated whole homogenates and 105,000 *g* soluble fractions were prepared as described previously [11]. Microsomal fractions were prepared by differential centrifugation. MCF-7/0 cells were suspended in 1.15% KCl (5×10^6–1×10^7 cells/mL) and then were lysed in an ice-bath by submitting them to sonication for a total of 10 sec (divided into 3 bursts). The lysates were subjected to centrifugation at 800 *g* and 4° for 20 min, and the resultant supernatant fractions were further centrifuged at 105,000 *g* and 4° for 60 min. The 105,000 *g* pellets thus obtained (microsomes) were washed once with water and resuspended in 200 μL of water by brief sonication (2 sec). These were the preparations used when cytochrome P450 IA1 activity was to be quantified.

Aldehyde dehydrogenase, glutathione *S*-transferase and DT-diaphorase activities were determined as described previously [13]. Substrates were benzaldehyde (4 mM), 1-chloro-2,4-dinitrobenzene (1 mM) and 2,6-dichlorophenol-indophenol (40 μM), respectively. The method of Burke *et al.* [16] was used to quantify cytochrome P450 IA1 activity. The reaction mixture (1 mL; pH 7.6) contained 100 mM potassium phosphate, 5 μM 7-ethoxyresorufin, 250 μM NADPH and microsomes. The reaction mixture (minus NADPH) was preincubated at 37°

Table 1. ALDH-3, DT-diaphorase, glutathione *S*-transferase and cytochrome P450 IA1 activities in untreated and catechol-treated human MCF-7/0 breast adenocarcinoma cells*

Enzyme	mIU/10^7 cells		Treated/Control
	Control	Treated	
NAD-ALDH-3	1.5 ± 0.1	381 ± 13	254
NADP-ALDH-3	1.7 ± 0.1	768 ± 43	452
DT-Diaphorase	77 ± 4	6395 ± 748	83
Glutathione *S*-transferase	24 ± 2	250 ± 11	10
Cytochrome P450 IA1	0.032 ± 0.003	0.034 ± 0.002	1

* Exponentially growing MCF-7/0 cells (1×10^5) were cultured in the presence of vehicle (control) or 30 μM catechol (treated) for 5 days. They were then harvested, and Lubrol-treated whole homogenates (2.5×10^4–5×10^6 cells) were prepared and assayed for aldehyde dehydrogenase, DT-diaphorase, and glutathione *S*-transferase activities, as described in Materials and Methods. In addition, microsomal fractions (2.5×10^6–1×10^7) were prepared and assayed for cytochrome P450 IA1 activity, as described in Materials and Methods. Each value is the mean ± SEM of four determinations.

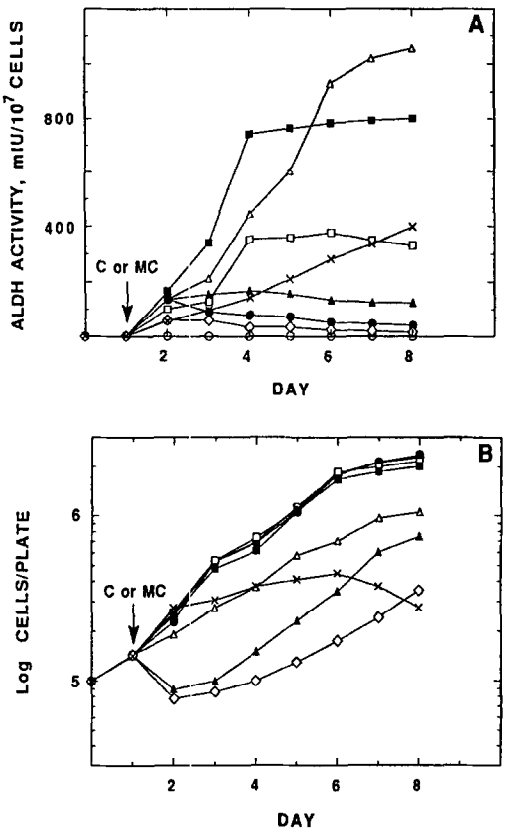

Fig. 1. Induction of aldehyde dehydrogenase (ALDH) activity in MCF-7/0 cells by catechol (C): concentration dependence. Exponentially growing MCF-7/0 cells were exposed continuously to vehicle (○), 3 μM 3-methylcholanthrene (MC) (×), or 3 (●), 10 (□), 30 (■), 60 (△), 100 (▲), or 300 (◇) μM catechol, for 7 days. Lubrol-treated whole homogenates of these cells were then prepared and ALDH activity therein (panel A) was quantified as described in Materials and Methods; benzaldehyde (4 mM) and NADP (4 mM) were used as substrate and cofactor, respectively. Growth of MCF-7/0 cells in the presence of vehicle, 3 μM 3-methylcholanthrene, or various concentrations of catechol is shown in panel B. Values are means of duplicate determinations made in each of two separate experiments.

for 2 min, NADPH was added, and the reaction (O-dealkylation of 7-ethoxyresorufin to resorufin) was monitored with the aid of an automated Spex-3000 spectrofluorimeter; excitation and emission wavelengths were 530 and 585 nm, respectively. Authentic resorufin was used to generate a standard curve. O-Dealkylation of ethyl ethers, e.g. 7-ethoxyresorufin, is preferentially catalyzed by cytochrome P450 IA1 as opposed to cytochrome P450 IA2 [16, 17], and it has been reported that, whereas polycyclic aromatic hydrocarbons induce cytochrome P450 IA1 activity in MCF-7/0 cells, they do not induce cytochrome P450 IA2 activity in these cells [18]. Hence, the assumption is that the O-dealkylation of ethoxyresorufin measured in this investigation was catalyzed by cytochrome P450 IA1.

Chromatographic purification of catechol-induced ALDH-3, protein determinations, SDS–PAGE, immunoblot analysis and data analysis were as described previously [11, 13].

Drug exposure and the colony-forming assay used to determine surviving fractions were as described previously [11–13]. Essentially, freshly harvested cells were diluted with drug-exposure medium to a concentration of 1×10^5 cells/mL and were then exposed to mafosfamide, phosphoramide mustard or vehicle for 30 min at pH 7.4 and 37° after which they were harvested and cultured in drug-free growth medium for 15 days. Colonies (≥50 cells) were then visualized with methylene blue dye and counted.

Isolation of total RNA from cultured cells, isolation of poly(A)$^+$-enriched RNA from total RNA with the aid of oligo(dT)-cellulose columns, resolution of poly(A)$^+$-enriched RNA on agarose gels (1%) containing formaldehyde, transfer of resolved RNA onto Zeta-Probe nylon membranes (Bio-Rad Laboratories, Richmond, CA), hybridization and autoradiographic visualization of the resultant blots were essentially as described by Angelini *et al.* [19] and Sambrook *et al.* [20]. Briefly, Zeta-Probe membranes containing poly(A)$^+$-enriched RNA were baked at 80° for 2 hr and placed in thermoresistant plastic bags. The bags were then filled with 10 mL hybridization solution, pH 7.2, containing 750 mM sodium chloride, 75 mM sodium citrate, 20 mM sodium phosphate, 7% SDS (w/v), 20% Denhardt's reagent (2 mg each of Ficoll, polyvinylpyrrolidone and bovine serum albumin) and 1 mg denatured salmon sperm DNA, sealed and prehybridized for 8 hr at 50°. Freshly prepared 5′-end-labeled ALDH-3 specific oligonucleotide probe was added to the hybridization bag, and incubation was continued overnight (~14 hr). Following hybridization, the membrane was washed twice at 50° for 30 min in a solution, pH 7.2, containing 100 mM sodium chloride, 10 mM sodium citrate, 25 mM sodium phosphate, 5% SDS and 20% Denhardt's reagent, and once at 25° for 30 min in a solution containing 30 mM sodium chloride, 3 mM sodium citrate and 1% SDS. The wet membrane was then wrapped in Saran wrap, and the wrapped membrane, juxtaposed to a Kodak X-OMAT-AR X-ray film, was sandwiched between intensifying screens for 72 hr. The oligonucleotide probe was removed from the membrane by incubating the latter at 95° for 8 hr in a solution containing 3 mM sodium chloride, 0.3 mM sodium citrate and 0.5% SDS. The membrane was then rehybridized with ^{32}P-labeled human β-actin cDNA as described above except that the incubations were at 68°. Films were developed in an automated X-ray film processor (model QX-130A plus, Konica Corp., Japan).

RESULTS AND DISCUSSION

ALDH-3 activity was elevated markedly when human breast adenocarcinoma MCF-7/0 cells were cultured in the presence of 30 μM catechol for 5 days (Table 1). DT-Diaphorase and glutathione *S*-transferase activities were also elevated but to a significantly lesser extent.

Given that the rat ALDH-3 gene, like the rat and human DT-diaphorase and the rat glutathione *S*-

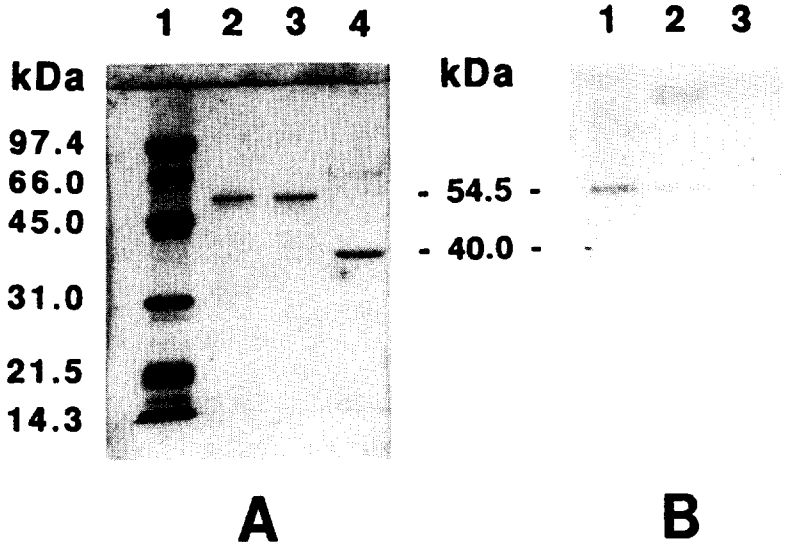

Fig. 2. ALDH-3 purified from catechol-treated MCF-7/0 cells: subunit molecular weight and recognition of the denatured enzyme by anti-stomach mucosa type-1 ALDH-3 IgY. Induction of ALDH-3 by catechol, and the subsequent purification of this enzyme, were as described in Materials and Methods and in Table 1. (Panel A) SDS–PAGE of molecular weight markers (lane 1) and 5 μg each of purified stomach mucosa type-1 ALDH-3 (lane 2), catechol-induced enzyme (lane 3), and MCF-7/OAP type-2 ALDH-3 (lane 4) was as described in Materials and Methods. Molecular weight markers were lysozyme (14.3 kDa), trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), BSA monomer (66 kDa) and phosphorylase *b* (97.4 kDa). Proteins in each lane were visualized by staining with Coomassie Brilliant Blue R-250. A plot of log M_r versus mobility was used as described in Materials and Methods to estimate the subunit molecular weight of the catechol-induced enzyme. (Panel B) Purified stomach mucosa type-1 ALDH-3 (lane 1), purified catechol-induced enzyme (lane 2), and purified MCF-7/OAP type-2 ALDH-3 (lane 3) were submitted to SDS–PAGE and electrotransferred onto an Immobilon-PVDF transfer membrane; attempted visualization of the denatured enzymes with anti-stomach mucosa type-1 ALDH-3 IgY was as described in Materials and Methods. Placed on the gel were 5 μg of each purified enzyme.

transferase Ya subunit genes [3–6, 8, 9], contains both XRE and ARE in its 5′-flanking region [7], it is likely that XRE, as well as ARE, are also present in the 5′-flanking region of the human ALDH-3 gene. XRE, but not ARE, are also found in the 5′-flanking region of the cytochrome P450 IA1 gene [reviewed in Refs. 21 and 22]. Induction of cytochrome P450 IA1 activity by 3-methylcholanthrene and other ligands for the Ah receptor is thought to be the result of a sequence of events culminating in the activation of cytochrome P450 IA1 gene transcription when the Ah receptor–ligand complex binds to the XRE present in the 5′-flanking region of this gene [reviewed in Refs. 21 and 22]. Induction of ALDH-3, DT-diaphorase and glutathione *S*-transferase activities by Ah receptor ligands is also thought to be via XRE, but, in addition, (induced) cytochrome P450 IA1 catalyzes the conversion of some of these ligands, e.g. 3-methylcholanthrene, to metabolites that induce the synthesis of these enzymes via ARE [reviewed in Refs. 1 and 2]. Cytochrome P450 IA1 activity was elevated markedly (>10-fold)*, as were ALDH-3, DT-diaphorase, and glutathione *S*-transferase activities [12, 13], when MCF-7/0 cells were cultured in the presence of 3 μM 3-methylcholanthrene for 5 days. Consistent with the notion that catechol induction of ALDH-3, DT-diaphorase and glutathione *S*-transferase activities is directly via ARE, cytochrome P450 IA1 activity was not elevated when MCF-7/0 cells were cultured in the presence of 30 μM catechol for 5 days (Table 1).

NAD(P)-dependent ALDH-3 activity was also elevated markedly when MCF-7/0 cells were cultured in the presence of other phenolic antioxidants for 5 days, viz. 30 μM of either hydroquinone (>150-fold), *tert*-butyl-hydroquinone (>50-fold), BHA (>50-fold), ethoxyquin (>50-fold), BHT (>15-fold), or Vitamin E (~5-fold) (data not shown). Moreover, it was elevated markedly when human breast adenocarcinoma MDA-MB-231 (>500-fold) and SK-BR-3 (~10-fold), human breast carcinoma T-47D (>500-fold) and ZR-75-1 (~5-fold), and human colon carcinoma HCT 116b (>50-fold) and colon C (~4-fold), cells were cultured in the presence of 30 μM catechol for 5 days (data not shown). This distinguishes phenolic antioxidant induction of ALDH-3 expression from that effected by Ah receptor agonists, e.g. polycyclic aromatic hydrocarbons such as 3-methylcholanthrene and 3,4-benzpyrene, since the latter, while inducing ALDH-

* Sreerama L, Rekha GK and Sladek NE, unpublished observations.

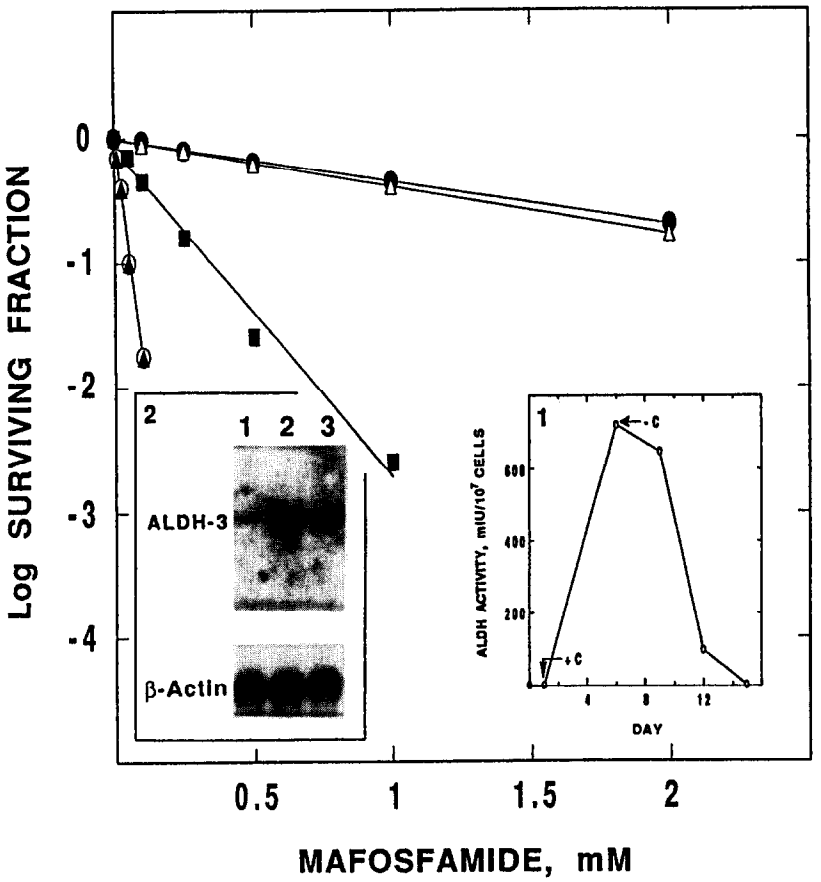

Fig. 3. Effect of adding and then removing catechol from the culture medium on the sensitivity of MCF-7/0 cells to mafosfamide. Exponentially growing MCF-7/0 cells were cultured in the presence of 30 μM catechol (C) for 5 days. At the end of this time, cells were harvested, washed, resuspended in catechol-free growth medium, and cultured for an additional 9 days. Sensitivity to mafosfamide was determined as described in Materials and Methods on days 0 (○), 6 (●), 9 (△), 12 (■), and 15 (▲). Each point is the mean of measurements on triplicate cultures. The LC_{90} values (concentrations of drug required to kill 90% of cells) obtained from these plots were 55 (○), >2000 (●), >2000 (△), 370 (■), and 55 (▲) μM. (Inset 1) Aldehyde dehydrogenase activity at corresponding time points. NADP (4 mM)-linked aldehyde dehydrogenase-catalyzed oxidation of benzaldehyde (4 mM) was quantified in Lubrol-treated whole homogenates, as described in Materials and Methods, at the times indicated. Reaction mixtures (1 mL) contained whole homogenates prepared from 1.5×10^5 to 1×10^7 cells. Each value is the mean of duplicate determinations. (Inset 2) Northern blot analysis of poly(A)$^+$-enriched RNA isolated from MCF-7/0 cells (lane 1), MCF-7/0 cells treated with catechol (30 μM for 5 days) (lane 2), and MCF-7/0 cells treated with catechol (30 μM for 5 days) and then cultured in the absence of catechol for 3 days (lane 3). Isolation of total RNA, isolation of poly(A)$^+$-enriched RNA, electrophoresis of poly(A)$^+$-enriched RNA, transfer of poly(A)$^+$-enriched RNA onto a Zeta-Probe nylon membrane, and probing of the blotted membrane with ^{32}P-labeled oligonucleotide specific for human stomach mucosa type-1 ALDH-3 and with full-length β-actin cDNA were described in Materials and Methods. Placed on the gels were 10 μg of poly(A)$^+$-enriched RNA in each case.

3 expression in MCF-7/0 [13], T-47D*, and ZR-75-1* cells, do not do so in MDA-MB-231, SK-BR-3, HCT 116b, or colon C cells [13, 14].

Induction of ALDH-3 in MCF-7/0 cells by catechol was concentration dependent (Fig. 1). Maximum induction was achieved at a concentration of about 60 μM. A measurable increase in enzyme activity was seen when the catechol concentration was as low as 3 μM. Induction of ALDH-3 activity with the phenolic antioxidant could be achieved without measurably inhibiting cellular proliferation, whereas it could not in the case of Ah receptor agonists [12], thus further distinguishing the two mechanisms of induction.

We have shown previously that ALDH-3s can be of two types, viz. type-1 and type-2 [11]. Type-1 is found in human stomach mucosa, colon C cells, MCF-7/0 cells and 3-methylcholanthrene-treated MCF-7/0 cells [11–14]. Type-2 is found in MCF-7/OAP cells [11]. The latter were generated by growing MCF-7/0 cells in the presence of gradually increasing concentrations of 4-hydroperoxycyclophosphamide

for several months after which time the cells stably expressed high levels of a variant (type-2) ALDH-3 [11, 23]. While the two enzymes share many physical and catalytic characteristics, they also differ in some regards. Thus, the subunit molecular mass of the type-1 enzyme is 54.5 kDa, whereas that of the type-2 enzyme is 40 kDa, and whereas anti-stomach mucosa type-1 ALDH-3 IgY recognizes the type-1 subunit, it does not recognize the type-2 subunit. As judged by these criteria, the ALDH-3 induced by catechol is of the type-1 variety (Fig. 2).

Associated with the marked increase in ALDH-3 expression induced by 30 μM catechol in MCF-7/0 cells was a marked decrease in sensitivity to the cytotoxic action of mafosfamide (Fig. 3). Resistance to mafosfamide was oxazaphosphorine-specific since catechol-treated MCF-7/0 cells were not resistant to phosphoramide mustard; LC_{90} values for untreated and catechol-treated cells were 900 and 1200 μM, respectively (data not presented). Resistance to mafosfamide was transient since sensitivity to mafosfamide, as well as ALDH-3 activity, returned to basal levels within 10 days when catechol was removed from the culture medium. Consistent with transcriptional activation of the ALDH-3 gene by catechol, Northern blot analysis showed that the cellular content of ALDH-3 mRNA was increased markedly when MCF-7/0 cells were grown in the presence of this agent, and that upon its removal, the mRNA level decreased (Fig. 3).

It has now been demonstrated that, in culture models, cytosolic ALDH-3-mediated oxazaphosphorine-specific resistance can be (a) constitutive [14], and (b) induced by exposing cells to gradually increasing concentrations of oxazaphosphorines for several months [11, 23], Ah receptor ligands for a few days [12, 13], or phenolic antioxidants (and, undoubtedly, other ARE activators) for a few days. Constitutive resistance is indefinite by definition. Oxazaphosphorine-induced resistance was long-term, if not indefinite, in the absence of the inducing agent. In contrast, resistance induced by Ah receptor ligands or phenolic antioxidants quickly disappeared when the inducing agent was removed from the culture medium.

The potential significance of "transient" resistance relative to therapeutic strategy is self-evident, but it is yet to be demonstrated that transient resistance to the oxazaphosphorines ever occurs clinically, much less that it is, at least on occasion, the consequence of elevated ALDH-3 levels induced by pharmacological and/or dietary/environmental agents. However, given that only small amounts of ARE activators are needed to induce ALDH-3 expression, and the abundance and widespread distribution of such agents in the diet [reviewed in Refs. 1 and 24], clinical induction of resistance effected by ARE activators would seem especially likely, and an ongoing investigation in our laboratory has yielded preliminary information consistent with this possibility. Especially intriguing is the possibility that, in those cases where resistance to the oxazaphosphorines is induced by ARE activators, sensitivity to these agents could be quickly restored by an appropriate dietary change.

Thus far, our investigations have focused on induction of ALDH-3-mediated oxazaphosphorine-specific resistance. However, it may be that phenolic antioxidants and other ARE activators, as well as Ah receptor ligands, induce, in fact, multidrug resistance and even collateral sensitivity since they "coordinately" induce a number of additional drug-metabolizing enzymes. This notion is more completely discussed in a previous publication [13].

Oxazaphosphorines are also known to be carcinogenic [25]. The cytotoxic and carcinogenic actions of these agents are, at a biochemically fundamental level, almost certainly mechanistically identical. Thus, phenolic antioxidant induction of ALDH-3-mediated resistance to the oxazaphosphorines may also be viewed as a model of cancer chemoprotection. Identification of cancer chemoprotective agents is currently being pursued extensively [26]. In that regard, the determination of ALDH-3, glutathione *S*-transferase and/or DT-diaphorase levels in MCF-7/0 cells, and/or the relative sensitivity of these cells to oxazaphosphorines, e.g. mafosfamide and 4-hydroperoxycyclophosphamide, before and after short-term exposure to candidate agents, may be of value in preliminarily identifying such agents rapidly and economically.

Acknowledgements—This work was supported by USPHS Grant CA 21737, Bristol-Myers Squibb Company Grant 100-R220, and DOA Grant DAMD 17-94-J-4057.

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