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Collateral sensitivity to gemcitabine (2′,2′-difluorodeoxycytidine) and cytosine arabinoside of daunorubicin- and VM-26-resistant variants of human small cell lung cancer cell lines

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Abstract

Multidrug resistance (MDR), characterized by a cross-resistance to many natural toxin-related compounds, may be caused either by overexpression of a drug efflux pump such as P-glycoprotein, (P-gP), multidrug resistance proteins MRP1–3, or BCRP/MXR or, in the case of DNA topoisomerase II active drugs, by a decrease in the enzymatic activity of the target molecule termed altered topoisomerase MDR (at-MDR). However, human small cell lung carcinoma (SCLC) cell lines showed a collateral sensitivity to 2′,2′-difluorodeoxycytidine (gemcitabine, dFdC) and 1-β-D-arabinofuranosylcytosine (ara-C). H69/DAU, a daunorubicin (DAU)-resistant variant of H69 with a P-gP overexpression, and NYH/VM, a VM-26 (teniposide)-resistant variant of NYH with an at-MDR, were both 2-fold more sensitive to gemcitabine and 7- and 2-fold more sensitive to ara-C, respectively. MDR variants had a 4.3- and 2.0-fold increased activity of deoxycytidine kinase (dCK), respectively. dCK catalyzes the first rate-limiting activation step of both gemcitabine and ara-C. In addition, deoxycytidine deaminase, responsible for inactivation of dFdC and ara-C, was 9.0-fold lower in H69/DAU cells. The level of thymidine kinase 2, a mitochondrial enzyme that can also phosphorylate deoxycytidine and gemcitabine, was not significantly different between the variants. These differences most likely caused an increased accumulation of the active metabolites (dFdCTP, 2.1- and 1.6-fold in NYH/VM and H69/DAU cells, respectively) and of ara-CTP (1.3-fold in NYH/VM cells). Ara-CTP accumulation was not detectable in either H69 variant. The pools of all ribonucleoside and deoxyribonucleoside triphosphates were at least 3- to 4-fold higher in the NYH variants compared to the H69 variants; for dCTP and dGTP this difference was even larger. The higher ribonucleotide pools might explain the >10-fold higher accumulation of dFdCTP in NYH compared to H69 variants. Since dCTP is low, H69 cells might not need a high ara-CTP accumulation to inhibit DNA polymerase. This might be related to the lack of ara-CTP in H69 variants. In addition, the increased CTP, ATP, and UTP pools in the MDR variants might explain the increased ara-CTP and dFdCTP accumulation. In conclusion, the MDR variants of the human SCLC cell lines were collaterally sensitive due to an increased dCK activity, and consequently an increased ara-CTP and dFdCTP accumulation.

Keywords: Gemcitabine; Cytosine arabinoside; Multidrug resistance; Deoxycytidine kinase; Thymidine kinase 2; Deoxycytidine deaminase; Ribonucleoside triphosphate

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Abbreviations: ara-C, 1-β-D-arabinofuranosylcytosine; dCDA, deoxycytidine deaminase; dCK, deoxycytidine kinase; dCyd, deoxycytidine; dFdC, 2′,2′-difluorodeoxycytidine; MDR, multidrug resistance; MRP, multidrug resistance-associated protein; P-gP, P-glycoprotein; SCLC, small cell lung carcinoma; TCA, trichloroacetic acid; TK, thymidine kinase; and VM-26, teniposide, epipodophyllotoxin 4′-demethyl-*O*-(4,6-*O*-2-thenylidene-β-D-glucopyranoside).

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1. Introduction

dFdC, a deoxycytidine analog, is active *in vivo* and *in vitro* against SCLC [1], while 27% of patients with SCLC respond to gemcitabine treatment [2]; the combination with etoposide is even more active [3]. ara-C, also a deoxycytidine analog, is only active against acute myeloid leukemia. Both gemcitabine and ara-C require phosphorylation, and are catalyzed by dCK to their monophosphates dFdCMP and ara-CMP [4] and subsequently to dFdCTP and ara-CTP. However, the mitochondrial thymidine kinase 2 (TK2) [5,6] can also use gemcitabine as a substrate as well as the natural nucleosides thymidine (TdR), dCyd, and deoxyuridine (UdR). This is in contrast to TK1, a cytosolic cell cycle-dependent enzyme, which does not use dCyd or gemcitabine [6]. dCK is feedback-inhibited by dCTP, possibly down-regulating the phosphorylation of gemcitabine and ara-C [7]. An increase in the dCK/TK2 activity ratio may lead to an increase in the phosphorylation of gemcitabine and ara-C [8]. Both gemcitabine and ara-C can be deaminated by dCDA [4], thereby preventing activation. dFdCTP is incorporated into both DNA and RNA, but incorporation into DNA is considered to be the major mechanism of cell death [9,10]. Ara-C in its active form, ara-C triphosphate (ara-CTP), is a potent inhibitor of DNA synthesis by inhibition of DNA polymerase [11]. The diphosphate of gemcitabine, dFdCDP, inhibits ribonucleotide reductase (RNR), potentially leading to a decrease of deoxynucleoside triphosphate (dNTP) pools, predominantly dATP and to a lesser extent dCTP, which can favor further dFdCTP incorporation into DNA [12,13]. Inhibition of CTP synthetase by dFdCTP may decrease CTP pools [14]. In addition, dFdCTP, but not ara-CTP, inhibits dCMP deaminase, leading to further potentiation of gemcitabine activation [13]. These aspects make gemcitabine different from ara-C.

Treatment of tumors with one of the natural toxin-derived drugs (e.g. daunomycin, etoposide, vincristine) may lead to cross-resistance to other drugs, commonly termed MDR. MDR is characterized by an increased efflux due to an overexpression of the plasma membrane drug efflux pumps P-gP and MRP or by an atypical multidrug resistance (at-MDR) [15–17]. Water-soluble antimetabolites such as gemcitabine and ara-C are not substrates, but are transported across cell membranes via facilitated nucleoside diffusion [18]. Overexpression of MRP is commonly seen in SCLC cell lines and may play a role in the response to therapy in patients [19–21]. The role of P-gP in SCLC is as yet undefined, though recent studies have indicated that it may be of deleterious prognostic significance in a subset of patients [22]. This is presumably also the case for MRP. at-MDR cells are cross-resistant to all topoisomerase II poisons such as etoposide and adriamycin (doxorubicin), while P-gP expression cells are also cross-resistant to the tubulin-targeting agents vincristine and taxol [17,23].

Collateral sensitivity confers an increased sensitivity to drugs after the development of resistance to another drug. Previously, collateral sensitivity to gemcitabine and ara-C was found in both anthracycline (daunorubicin)- and topoisomerase II inhibitor (VM-26, teniposide)-resistant MDR variants of human SCLC cell lines [24]. We selected two of these variants to clarify the mechanism behind this increased sensitivity.

2. Materials and methods

2.1. Chemicals

dFdC was supplied by Eli Lilly Research Labs. Ara-C was obtained from Upjohn. RPMI was purchased from Flow Laboratories. Fetal bovine serum was from GIBCO, and TCA and gentamicin from Merck. [5-^{3}H]dCyd (744 GBq/mmol), [methyl-^{3}H]TdR (925 GBq/mmol), [8-^{3}H]deoxyadenosine-5′-triphosphate (3.4 Ci/mmol), and [methyl-^{3}H]thymidine-5′-triphosphate (30 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. All other chemicals were of analytical grade and commercially available.

2.2. Cell culture

OC-NYH (NYH) and NCl-H69 (H69) are the parental human SCLC cell lines [25,26] that were made resistant to VM-26 and daunorubicin by continuous exposure to low concentrations of these drugs, resulting in NYH/VM and H69/DAU, respectively [27,28]. Both NYH/VM and H69/DAU show the at-MDR phenotype with a decrease in topoisomerase II enzymatic activity. In addition, H69/DAU overexpresses P-gP but not MRP. All four cell lines were routinely cultured in RPMI supplemented with 10% fetal bovine serum, 1 mM HEPES, and 250 ng/mL of gentamicin. NYH and NYH/VM were cultured as semiattached cells and H69 and H69/DAU in suspension. All populations were cultured at 37° in a humidified atmosphere of 95% air and 5% CO_2 and regularly screened for *Mycoplasma* contamination by using a rapid detection system with a ^{3}H-labeled DNA probe (Gen-Probe) and were found to be negative.

2.3. Chemosensitivity testing

Sensitivity of the cells was determined by clonogenic assay as described previously [24]. Briefly, single-cell suspensions of 1–4. 10^6 cells were plated in soft agar on a feeder layer containing sheep red blood cells in Petri dishes. Three concentrations of both drugs and a control, all in triplicate, were tested. The number of cells was adjusted to obtain 2000–3000 colonies in the control dishes. After 14–21 days, the colonies were counted on an image analysis system. Colonies larger than 50 μm in diameter were regarded as positive. The dose reducing the number of colonies to 50% of control (LD_{50}) was determined from three

drug concentration points in linear regression analysis on logarithmically transformed response data.

2.4. Cell extraction and TK/dCK activity assay

Cell extraction and the TK/dCK assay were performed as described previously [8]. Briefly, 3.5×10^8 NYH cells, 3.4×10^8 NYH/VM cells, 1.0×10^8 H69 cells, and 2.0×10^8 H69/DAU cells were suspended in 20 mM potassium phosphate buffer (pH 7.4), sonificated, and centrifuged at 20,000 *g* for 10 min. The supernatant was fractionated with ammonium sulfate in two steps, followed by centrifugation, whereafter the pellet was resolved in a buffer containing 25 mM Tris–HCl, 5 mM $MgCl_2$, 15% glycerol, and 2 mM dithiothreitol (DTT, pH 7.5), and desalted by passage over a Sephadex G-25 column. Five-milliter fractions were collected and the bound proteins were chromatographed on a diethylaminoethyl ion-exchange column with a linear 0–0.5 M gradient of KCl. By this procedure TKI, which is positively charged under these conditions, is washed out with the unbound proteins in the first fractions. To measure dCK and TK activity, 20 μL of each 3 mL-fraction was mixed with a reaction mixture to final concentrations of 30 mM Tris–HCl, 1.5 mM ATP, 1.5 mM $MgCl_2$, 1.8 mg/mL of BSA, 3 mM NaF, 0.3 mM CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate), and 6.6 μM [^{3}H]dCyd or [^{3}H]TdR and incubated for 3, 6, and 9 min at 37°, whereafter the samples were spotted on DE-81 paper and washed in ammonium formate. Nucleotides were eluted by 0.5 mL of 0.2 M KCl/0.1 M HCl solution, scintillation liquid was added, and radioactivity then estimated.

2.5. dCDA enzyme activity assay

Activity of dCDA was determined as described earlier [29]. Briefly, 10,000 g supernatants were prepared and enzyme activity was determined at 37° in 3×10^6 cells with 500 μM dCyd as a substrate. After a 30- or 60-min incubation, the reaction was terminated by precipitating the proteins by addition of 40% w/v TCA. The supernatant was neutralized with trioctylamine/1,1,2-trichlorotrifluoroethane (v/v: 1/4). The upper, aqueous layer containing the substrate dCyd and the product deoxyuridine (UdR) was analyzed using reversed-phase HPLC [29].

2.6. dFdCTP, ara-CTP, and ribonucleoside triphosphate pools

Triphosphate accumulation, retention, and NTP pools were measured as previously described [30,31]. Briefly, 3×10^6 cells/5 mL of medium were plated in 25-cm^2 culture flasks (Costar), and drugs were added (dFdC final concentration of 1 μM to NYH and NYH/VM cells and 10 μM to H69 and H69/DAU cells; ara-C to a final concentration of 10 μM to all cell lines) and incubated for 24 hr. For the accumulation assay, cells were harvested after incubation. For the retention assay, cells were washed and incubated in fresh medium for 4 hr, then harvested. In order to extract the nucleotides, proteins were precipitated with 40% TCA and neutralized as described above. Finally, dFdCTP and NTPs were analyzed on HPLC using a Partisphere SAX (Whatman) column with a linear gradient between 5 mM $NH_4H_2PO_4$ (pH 2.8; buffer A) and 0.5 M $NH_4H_2PO_4$/0.25 M KCl (pH 3.0; buffer B; 35–100% B over 30 min) (30). For ara-CTP and NTP determination, isocratic anion-exchange HPLC was used (0.25 M KH_2PO_4 containing 0.5 M KCl (pH 4.5). Nucleotides were detected at 254 and 280 nm [31].

2.7. Deoxyribonucleotide extraction and measurement

dNTPs were extracted in a similar manner to NTPs, as described previously [30]. Before the assay, the TCA extract was dried using a Speedvac drying system at room temperature and, if necessary, stored at −20° until analysis. The assay for dNTP was based on the original DNA polymerase assay [32], optimized by the use of 96-well plates [33] and tailor-made oligonucleotides [34,35], and was performed as previously described for dCTP [36]. Dried samples were reconstituted in assay buffer (200 mM HEPES, 20 mM $MgCl_2$; pH 7.3) to a final concentration equivalent to 10^7 cells/mL. Samples and standards of 0, 1, 2.5, and 5 pmol of each dNTP were added to diethylaminoethyl (DEAE) filter plates (Millipore). To all wells, demi water was added to equalize the volume at 30 μL followed by 70 μL of a reaction mix, consisting of 10 μL [8-^{3}H]dATP (25 μM; 1.6 Ci/mmol; 0.04 μCi/μL) for detection of dCTP, dTTP, and dGTP, 10 μL [CH_3-^{3}H]dTTP (25 μM, 30 Ci/mmol, 0.04 μCi/μL) for dATP detection, 5 μL T-P mix, 5 μL Klenow DNA pol I, and 50 μL assay buffer. The filterplates were gently vortexed and incubated at room temperature for 2 hr. Each well was washed 4 times (0.25 M KH_2PO_4, 0.5 M KCl; pH 4.3), the blotted filters were punched out in liquid scintillation counting (LSC) vials, using a Multiscreen™ Assay System as described [36], and 500 μL 2M NaOH was added to each vial, followed after 3-hr shaking by 4.5 mL LSC fluid (Ultima Gold, Packard). Samples were counted in an LSC counter (Packard, 1900 TR).

2.8. Statistical analysis

Differences in LD_{50} values between parental cells and drug-resistant variants were evaluated using a Mann–Whitney U test. The computer program SPSS (version 7.5, SPSS, Inc.) was used for statistical analysis.

3. Results

3.1. Sensitivity to gemcitabine and ara-C

Table 1 summarizes the LD_{50} values of the clonogenic assay of the four cell lines for gemcitabine and ara-C after

Table 1
Sensitivities of the human SCLC cell lines to dFdC and ara-C

Drug	Cell line			
	NYH	NYH/VM	H69	H69/DAU
dFdC	1.35 ± 0.26	0.65 ± 0.12	1.73 ± 0.26	0.97 ± 0.08
ara-C	47.7 ± 2.30	24.7 ± 4.20	196.7 ± 27.7	27.7 ± 1.30

Drug sensitivity determined by clonogenic assay for 2–3 weeks. LD_{50} values are means in nM ± SEM of 4–8 experiments. Significance of differences in sensitivity between parental and MDR cells to dFdC and ara-C was analyzed by Mann–Whitney U tests: dFdC and ara-C on NYH vs NYH/VM, $P = 0.016$ and $P = 0.0007$, respectively; dFdC and ara-C on H69 vs H69/DAU, $P = 0.03$ and $P = 0.0003$, respectively.

continuous exposure. The parental H69 cells were 4-fold less sensitive to ara-C than NYH cells. NYH/VM cells were 2-fold more sensitive to both gemcitabine and ara-C than NYH cells, while H69/DAU cells were 2- and 7-fold more sensitive to gemcitabine and ara-C than their parental cells, respectively. The differences in sensitivity to gemcitabine and ara-C between parental and MDR cells were all significant.

3.2. Activities of dCK, TK, and dCDA

Since dCK plays an essential role in the activation of both gemcitabine and ara-C, we measured its activity in an attempt to gain insight into the increased sensitivity of the derived cell lines. Since TK2 plays an important role in dCyd metabolism, we also included TK2 (Fig. 1). The dCK activity of NYH/VM cells was 4.3-fold higher than that of its parental NYH cells. The TK2 activity with dCyd was 1.9-fold higher in NYH/VM cells. The dCK/TK2 ratio was 2.2-fold higher in NYH/VM than in NYH cells. In H69/DAU cells, dCK activity was 2.0-fold higher than in H69

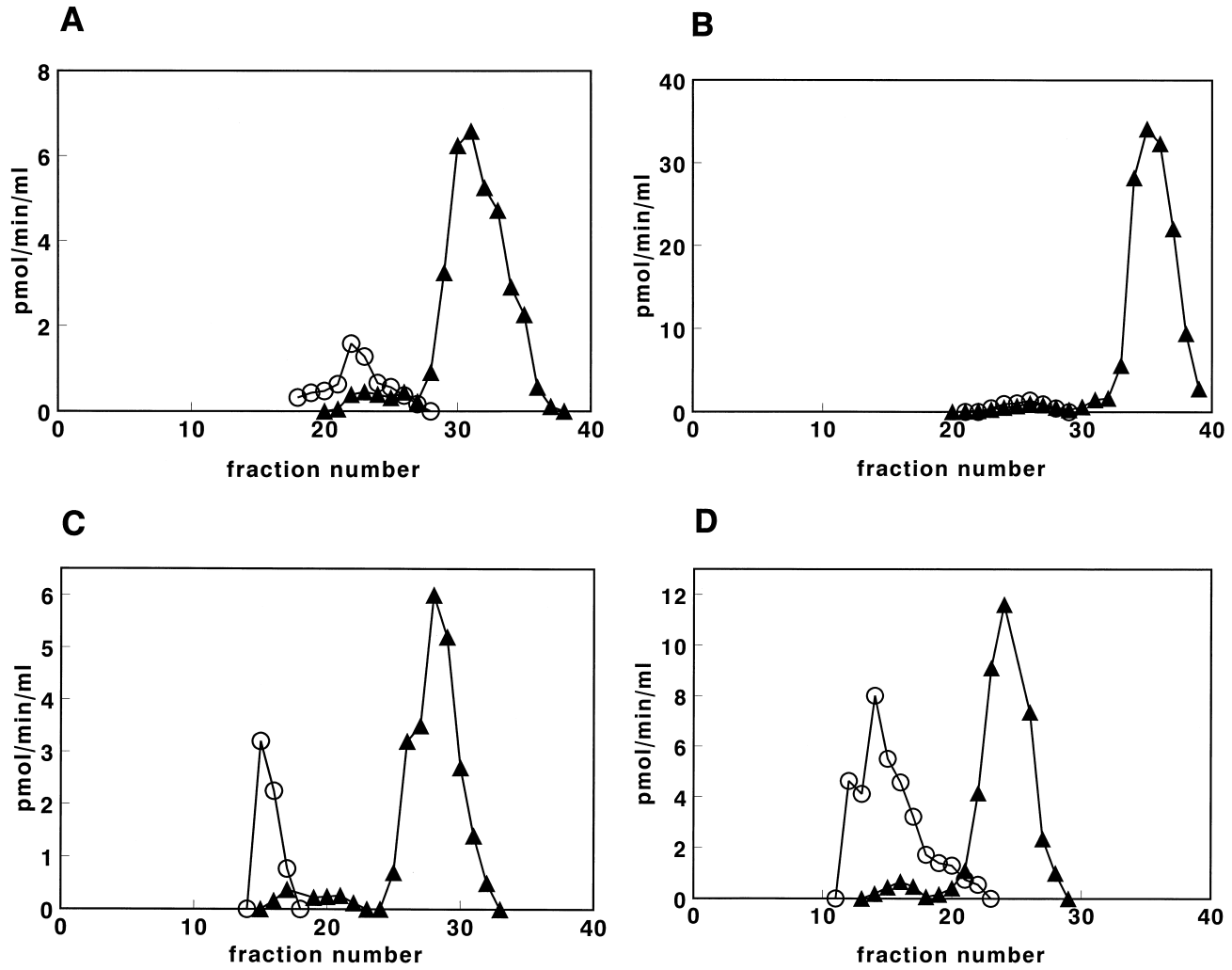

Fig. 1. Representative DEAE profiles of desalted ammonium sulfate-precipitated cell homogenate of 10^8 human SCLC cells. Thymidine kinase 2 (_○_) and deoxycytidine kinase (_▲_) activity. NYH (A) and its VM-26-resistant MDR variant NYH/VM (B); the human SCLC cancer cell lines H69 (C) and its daunorubicin-resistant MDR variant H69/DAU (D). The dCK activity in the eluents of NYH, NYH/VM, H69, and H69/DAU cells was 56.4, 241, 42, and 83 pmol/hr/10^6 cells, the TK2 with dCyd as a substrate 3.7, 7.2, 2.4, and 1.5, and the TK2 activity with thymidine as a substrate 11, 9, 12, and 64, respectively. TK1 elutes with the unbound proteins with the first fractions, well before dCK and TK2.

Table 2
Accumulation of dFdCTP and ara-CTP in the human SCLC cell lines NYH and H69 and their MDR variants NYH/VM and H69/DAU, respectively

Cell line	dFdCTP	ara-CTP
NYH	795 ± 66	478 ± 57
NYH/VM	1704 ± 351	609 ± 67
H69	58 ± 12	ND
H69/DAU	93 ± 23	ND

Values are means ± SEM of 3 experiments in pmol/10^6 cells. NYH and NYH/VM cells were exposed to 1.0 μM dFdC or 10 μM ara-C, and H69 and H69/DAU cells were exposed to 10 μM dFdC or 10 μM ara-C for 24 hr. ND, not detectable.

cells, but TK2 activity was lower, which resulted in a 3.2-fold higher dCK/TK2 ratio in H69/DAU than in H69 cells. TK2 activities with thymidine (TDR) as a substrate were comparable in both parent lines, and did not increase in NYH/VM cells; in H69/DAU cells, TK2 activity with TdR was 5-fold increased.

The parental SCLC cell line NYH had an 8.2-fold higher dCDA activity than the H69 cell line (2.21 vs 0.27 nmol/hr/10^6 cells, respectively). In NYH/VM cells, the dCDA activity (4.08 nmol/hr/10^6 cells) was 1.8-fold higher than that of its parental cells, but in H69/DAU cells dCDA activity (0.03 nmol/hr/10^6 cells) was 9-fold lower than in H69 cells.

3.3. dFdCTP and ara-CTP accumulation and retention

dFdCTP and ara-CTP, the active metabolites of gemcitabine and ara-C, were measured after 24-hr exposure to the drugs (Table 2). dFdCTP accumulation was higher than ara-CTP accumulation in all cell lines, while the more sensitive NYH cells accumulated more ara-CTP and dFdCTP than H69 cells. The MDR variant NYH/VM accumulated 2.1- and 1.3-fold more dFdCTP and ara-CTP than its parental cell line, NYH, respectively. Both H69 and H69/DAU cells accumulated dFdCTP to a low extent, while ara-CTP pools could not be determined, despite the use of a higher drug concentration and more cells. H69/DAU accumulated 1.6-fold more dFdCTP than H69. After removal of the drugs, dFdCTP and ara-CTP pools did not change in NYH cells, while in NYH/VM cells the pools increased 1.3- and 1.2-fold, respectively. In H69 and H69/DAU cells, dFdCTP pools increased 3- and 1.3-fold, respectively.

3.4. Ribonucleoside and deoxyribonucleoside triphosphate pools

Since both NTP and dNTP pools play a role in gemcitabine and ara-C cytotoxicity, we measured these pools in the four cell lines (Table 3). Both the NTP and dNTP pools were at least 2-fold higher in the parental NYH cells than in the parental H69 cells ($P < 0.05$), while dCTP and dGTP were even 10-fold higher in NYH cells ($P \leq 0.02$). As expected, dNTP concentrations were much lower than the NTP pools, but in the MDR variants this difference was more pronounced. In addition, significant ($P < 0.05$) differences between the parent and MDR variants were found in both pools: all NTP pools were 1.4- to 1.8-fold higher in the MDR variant.

Gemcitabine exposure showed the most pronounced effect on NTP pools (Fig. 2A). In both NYH variants, gemcitabine increased ATP, UTP, and GTP pools 1.7- to 2.3-fold, while in parent H69 a 1.5-fold increase was found for all nucleotides. In H69/DAU cells, gemcitabine only caused a moderate 1.2-fold increase in CTP pools.

The effect of ara-C was limited (Fig. 2B): in NYH cells all nucleotides increased about 1.8-fold, while in H69 cells ara-C only increased ATP and GTP pools about 1.5-fold. Ara-C did not affect nucleotide pools in the MDR variants in a significant manner. In NYH/VM cells, ara-C increased ATP and GTP pools 1.6- and 1.5-fold, respectively, while in H69/DAU cells only CTP pools increased 1.2-fold during dFdC exposure.

4. Discussion

In this paper, we describe that the collateral sensitivity to gemcitabine of multidrug-resistant human SCLC cell lines

Table 3
Ribonucleoside and deoxyribonucleoside triphosphate pools of untreated human SCLC cell lines NYH and H69 and their MDR variants NYH/VM and H69/DAU, respectively

	NYH	NYH/VM	H69	H69/DAU
CTP	773 ± 100	1198 ± 179	201 ± 21	350 ± 69
UTP	2820 ± 327	4274 ± 762	694 ± 64	1116 ± 197
ATP	5983 ± 753	8378 ± 1161	2154 ± 325	3197 ± 533
GTP	1141 ± 100	2043 ± 289	505 ± 61	696 ± 101
dCTP	10.1 ± 1.2	9.4 ± 1.7	0.7 ± 0.4	5.0 ± 2.1
dTTP	17.1 ± 3.1	17.8 ± 6.2	3.4 ± 2.6	5.0 ± 4.0
dATP	6.7 ± 0.6	9.2 ± 0.6	1.7 ± 0.9	3.3 ± 1.8
dGTP	3.8 ± 0.7	4.0 ± 0.7	ND	0.8 ± 0.8

Values are means ± SEM of 3 experiments in pmol/10^6 cells. ND, not detectable.

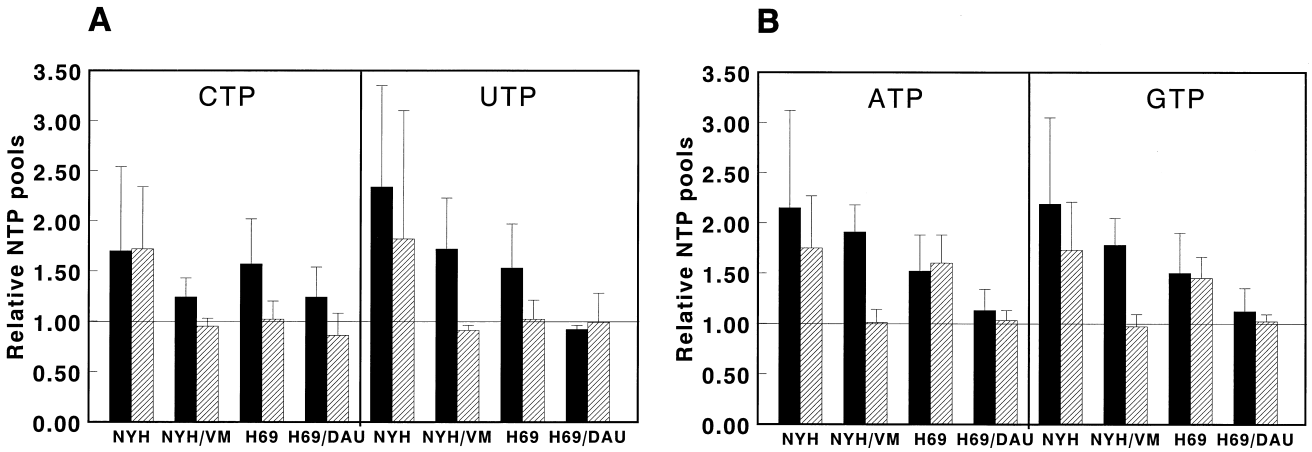

Fig. 2. Change in CTP and UTP pools (A) and ATP and GTP pools (B) of the human SCLC cell line NYH, its VM-26-resistant MDR variant NYH/VM, the human SCLC cancer cell line H69, and its daunorubicin-resistant MDR variant H69/DAU during 24-hr exposure to dFdC and ara-C. Exposure to 1 μM (NYH, NYH/VM) or 10 μM (H69, H69/DAU) dFdC (■) and 10 μM (all cell lines) ara-C (▨). Values are means ± SEM of 3 experiments. Basal ribonucleotide pools as given in Table 3 were set at 1.

was associated with an increased dCK activity, which may be the major alteration. The two pairs of cell lines were chosen from a larger panel showing collateral sensitivity to either gemcitabine or ara-C [24]. These MDR cell lines also had a broad cross-resistance to various drugs such as tubulin inhibitors (e.g. vincristine, taxol) and topoiosomerase II inhibitors (e.g. doxorubicin, etoposide). The cross-resistance to topo II inhibitors was probably related to a down-regulation of topo IIα in NYH/VM and H69/DAU [27,28], while overexpression of P-gP might explain cross-resistance to the taxanes and anthracyclines (e.g. daunorubicin) in H69/DAU cells, but not in NYH/VM cells, which did not have a P-gP overexpression [37].

Previous studies on the ara-C sensitivity of MDR cells showed variable results, while data on gemcitabine were not available. Human erythroleukemia K562 cells transfected with human *mdr* cDNA did not show a change in ara-C sensitivity, but were resistant to classic MDR agents [38], similarly to a CHO line reconstructed with proteoliposomes containing a partially purified P-gP [39]. However, a cross-resistance to ara-C was found in a daunorubicin-resistant variant of the human erythroleukemia cell line K562, associated with decreased dCK activity [40]. In contrast, ara-C sensitivity was increased in refractory AML cells with P-gP or MRP overexpression after treatment of these patients with MDR drugs [41]. This treatment might have selected P-gP- or MRP-overexpressing cells with an altered enzymatic profile favorable to cell survival. Increased dCK might be part of this.

Recently, it was shown that cytotoxic stress due to inhibition of DNA synthesis by 2-chloro-2′-deoxyadenosine (Cl-Ado) resulted in a rapid, transient rise in dCK activity, possibly due to an increased activation through a posttranslational modification of the enzyme [42]. This transient activation might be part of the cellular restoration process occurring after drug treatment. However, the increase in dCK activity found in SCLC cell lines was persistent, and remained so after culturing in drug-free medium.[1]

The increased dCK activity in both NYH/VM and H69/DAU and the decreased dCDA activity in H69/DAU might explain the enhanced dFdCTP and ara-CTP accumulation. The favorable substrate specificity for dCK of gemcitabine compared to ara-C [4] might partially explain the higher sensitivity to gemcitabine. In all cells, the TK2 activity is very low and may not contribute to either gemcitabine or ara-C phosphorylation. This may also be due to the low substrate specificity of gemcitabine and especially ara-C for TK2 [43].

Intrinsic CTP, UTP, and ATP pools might play an important role in dFdCTP accumulation in solid tumor cell lines [44], while dCTP plays an important role in feedback regulation of dCK [13]. In addition, appropriate dNTP pools are required for DNA synthesis and repair. In particular, gemcitabine has a profound effect on nucleotide pools [45], especially in terms of an increase in ATP and UTP. This might partially explain the higher accumulation of dFdCTP compared to ara-CTP, because ATP and UTP are regulators and phosphate donors for deoxynucleoside phosphorylation [44,46]. Since cells were continuously exposed to either gemcitabine or ara-C in the cytotoxicity experiments, the increased accumulation of dFdCTP and ara-CTP seems the major determinant in the enhanced sensitivity to these drugs. Retention may not play a role under these conditions. Ara-CTP accumulation was not detectable in either H69 variant despite changing incubation conditions. Considering the cytoxicity, ara-CTP should be formed albeit at low undetectable levels. Interestingly, the pyrimidine dCTP and dTTP levels were also very low. This might mean that due to a decreased competition with the normal dNTP, a low

[1] Bergman AM, Munch-Petersen B, Peters GJ. Unpublished data.

ara-CTP concentration may be sufficient to inhibit DNA polymerase.

In conclusion, the MDR variants of two parental SCLC cell lines were collaterally sensitive to both gemcitabine and ara-C, possibly due to an increased dCK activity in both variants, as well as to a decreased dCDA activity in H69/DAU cells. This led to an increased dFdCTP and ara-CTP accumulation.

Acknowledgments

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