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Deoxycytidine Kinase and Deoxycytidine Deaminase Activities in Human Tumour Xenografts

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Deoxycytidine kinase (dCK) and deaminase (dCDA) are both key enzymes in the activation and inactivation, respectively, of several deoxycytidine antimetabolites. We determined the total dCK and dCDA activities using standard assays, in 28 human solid tumours grown as xenografts in nude mice, and four corresponding cell lines. dCK activities in colon tumours varied from 11 to 12 nmol/h/mg protein, in ovarian tumours from 3 to 10 nmol/h/mg protein, in soft tissue sarcomas from 2 to 7 nmol/h/mg protein and in squamous cell carcinomas of the head and neck about 45-fold, between 0.4 and 18 nmol/h/mg protein. The dCDA activities showed a larger variation, from 243 to 483, 14 to 1231, 3 to 7 and 1 to 222 nmol/h/mg protein, respectively. The ratios of dCK vs. dCDA activities in these tumours varied from 0.025 to 0.046, 0.004 to 0.240, 0.581 to 1.123 and from 0.012 to 4.227, respectively. In four cell lines (A2780, OVCAR-3, WiDr and UM-SCC-14C), sources for some of the abovementioned tumours, a different pattern in dCK and dCDA was observed than in the corresponding tumours. The variation in dCDA activities was in a smaller range (20-fold) than in the tumours (40-fold). In all cell lines dCK activity was higher than dCDA activity, in contrast to the corresponding tumours, in which the reverse pattern was observed. Previously, some of the tumours were tested for sensitivity to the deoxycytidine analogues 5-aza-deoxycytidine and 2′,2′-difluorodeoxycytidine. In the sensitive tumours, both the highest and lowest dCK activity was observed, indicating that dCK activity in solid tumours is high enough to activate deoxycytidine analogues.
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INTRODUCTION

THE ENZYME deoxycytidine kinase (NTP: deoxycytidine 5′-phosphotransferase, EC 2.7.1.74) (dCK) plays an essential role in the activation of several antiviral and anticancer deoxynucleosides. Examples are the cytidine analogues 2′,3′-dideoxycytidine [1], 1-β-D-arabinofuranosyl cytosine (ara-C) [2–4], 2′,2′-difluorodeoxycytidine (dFdC) [3, 4] and 5-aza-2′-deoxycytidine (5-aza-CdR) [5]. Also, purine analogues such as 2-chloro-2′-deoxyadenosine and the normal nucleosides deoxyadenosine and deoxyguanosine are good substrates [6]. dCK catalyses the rate-limiting phosphorylation of deoxycytidine (CdR) and its analogues to their corresponding monophosphates. Subsequent phosphorylation yields triphosphates, which can be incorporated into DNA [1–6]. Deoxycytidine deaminase (EC 3.5.4.5) (dCDA) catalyses the inactivation of cytidine (CR), CdR and its analogues to their deaminated products [2, 4, 7, 8]. The presence of both enzymes has been demonstrated in a variety of human tissues and tumour cells, and has been characterised most extensively in leukaemic cells. dCK is present in resting and proliferating peripheral blood lymphocytes, leukaemic T lymphoblasts, monocytes and macrophages and in a variety of normal tissues [9–11].

dCDA has been detected in liver, kidney, spleen, heart and muscle tissue, as well as in chronic myelogenous leukaemic cells, peripheral white blood cells and bone marrow [7, 8, 10]. Both enzymes have been shown to exhibit tissue- and species-dependent activities [10–12]. Information about the enzymes in solid malignancies is scarce [10, 13]. Knowledge about the properties of both enzymes in solid tumours may contribute to a better application of deoxynucleoside analogues for the treatment of cancer. For this purpose we have determined the total dCK activity and that of dCDA in human tumour xenografts from various histological origins: ovarian carcinomas, soft tissue sarcomas, colon carcinomas and squamous cell carcinomas of the head and neck (HNSCC). For comparison we have also determined dCK and dCDA activities in four cell lines, each corresponding to one of the tumours.

MATERIALS AND METHODS

Materials

CdR and deoxyuridine (UdR) were purchased from Sigma Chemical Co. (St Louis, Missouri, U.S.A.). Deoxy-[-5^{3}H]-cytidine (^{3}H-CdR, 21.5 Ci/mmol) was obtained from Amersham International (Buckinghamshire, U.K.). All other chemicals were of analytical grade and are commercially available.

Tumours

Tumours from four different histological types, routinely grown in this laboratory, were used for enzyme activity measure-

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ments: colon carcinomas, ovarian carcinomas, HNSCC and soft tissue sarcomas. In Table 1, some characteristics of the tumours are summarised.

Tumours were implanted subcutanously in athymic female nude mice, as described previously [14, 15]. At a diameter of 5–10 mm the tumours were excised and immediately frozen in liquid nitrogen and stored at −70°C. Under these conditions the enzymes were stable for several years.

Cell lines

In the following cell lines, used for raising tumours, we determined the activities of dCK and dCDA: the colon carcinoma cell line WiDr, the ovarian carcinoma cell lines OVCAR-3 and A2780 and the HNSCC cell line UM-SCC-14C. Cells were cultured in Dulbecco's modification of Eagle's medium (Gibco BRL, Paisley, U.K.), supplemented with 5% v/v heat inactivated fetal calf serum (Gibco BRL), 1 mmol/l L-glutamine (Sigma, St Louis, Missouri, U.S.A.) and 250 ng/ml gentamicin, at 37°C in 5% CO_2. Cells were harvested 2 days after subculture, when still in exponential growth. After trypsinisation, cells were washed with phosphate-buffered saline and counted. The cell pellets were immediately frozen in liquid nitrogen and stored at −70°C.

dCK and dCDA assays

General. A minimum of 0.5 g of tumour was required for the two assays. Frozen tissues were pulverised using a microdismembrator (B. Braun, Melsungen, Germany), as described previously [27]. The powder was reconstituted in dCK buffer (0.3 mol/l Tris–HCl, 50 μmol/l β-mercaptoethanol, pH 8.0) at 1 g tumour per 3 ml buffer. The suspension was centrifuged for 10 min at 2800 *g* at 4°C. The supernatant was subsequently centrifuged for 20 min at 10 000 *g*. This second supernatant was immediately used for the enzyme assays. Dialysis of the supernatant did not result in enzyme activities different from those in non-dialysed samples, so this procedure was omitted. Dilutions of the supernatant for the dCK assay were made in dCK buffer, and for the dCDA assay in dCDA buffer (0.1 mol/l Tris–HCl, 50 μmol/l β-mercaptoethanol, pH 8.0). One part of the undiluted supernatant was taken for determining its protein content, using the Biorad Bradford protein assay [28]. Enzyme activities were expressed as nmol product formed per hour per mg protein (nmol/h/mg protein).

For determination of enzyme activities in cell lines, a minimum of 25×10^6 cells were required. The pellet was thawed and resuspended in cold dCK buffer to a concentration of

Table 1. Characteristics of the tumours used for determination of dCK and dCDA activities

Tumour	Histology*	DT*	Reference
Colon			
LS174T	Mod. diff. mucinous adenocarcinoma	4	16,17
SW1398	Mod./well diff. mucinous adenocarcinoma	4	16
WiDr	Poorly diff. mucinous adenocarcinoma	8	18
Ovarian			
A2780	Undiff. ovarian carcinoma	6	14,19
Ov. Pe	Mod. diff. mucinous adenocarcinoma	8	14
Ov. He	Mod. diff. mucinous adenocarcinona	17	14
OVCAR3	Poorly/mod. diff. serous adenocarcinoma	8	14,20
Ov. Ri (C)	Mod. diff. serous adenocarcinoma	11	14
FKo	Mod. diff. serous adenocarcinoma	12	14
Sarcomas			
S. Ho	Malignant fibrous histiocytoma	6	21
S. Hu	Leiomyosarcoma	12	21
S. La (C)	Malignant fibrous histiocytoma	13	21
S. To	Synovial sarcoma	13	21
HNSSC			
HNX-SK	Mod. diff. tongue ssc	nd	22
HNX-14A	Poorly diff. oral cavity ssc	16	15
HNX-14C	Poorly diff. ssc metastasis of oral cavity 14A	7	15
HNX-W	Mod. diff. oral cavity ssc	9	23
HNX-LP	Mod. diff. oral cavity ssc	9	23
HNX-HE	Mod. diff. oral cavity ssc	nd	24
HNX-E	Mod. diff. oral cavity ssc	nd	25
HNX-HN	Poorly diff. oropharynx ssc	6	15
HNX-DU	Undiff. hypopharynx carc.	4	25
HNX-OO	Poorly diff. hypopharynx ssc	nd	—
HNX-FR	Mod. diff. hypopharynx ssc	nd	26
HNX-FA	Mod. diff. hypopharynx ssc	nd	26
HNX-22B	Well diff. hypopharynx carc.	11	15
HNX-KE	Poorly diff. larynx ssc	6	23
HNX-HEp-2	Poorly diff. larynx ssc	8	26

*All tumours were from human origin. Mod., moderately; (un)diff., (un)differentiated; ssc, squamous cell carcinoma; DT, mean tumour volume doubling time in days, as determined in our laboratory; nd, not determined.

30×10^6 cells/ml. The suspension was sonicated while chilled on ice, five times for 5 s, with 5 s rest between cycles. Then the suspension was centrifuged for 20 min at 10 000 *g*. The supernatant was immediately used in the enzyme assays.

dCK assay

To 25 μl of enzyme containing supernatant (2–14 μg protein/μl) 25 μl of substrate mixture were added. The substrate mixture was prepared by mixing 2 volumes of Mg-ATP (50 mmol/l ATP in 25 mmol/$MgCl_2$, pH 7.4), 2 volumes ^{3}H-CdR (0.044 Ci/mmol, 1.14 mmol/l) and one volume of dCK buffer. This reaction mixture was incubated for 15–60 min at 37°C. The reaction was terminated by heating at 95°C for 3 min and by the subsequent addition of 10 μl 5 mmol/l unlabelled CdR. The denaturated protein was precipitated by centrifugation for 5 min at 12 000 *g*. The substrate (CdR) was separated from the product deoxycytidine monophosphate (dCMP) by thin layer chromatography on polyethylene imine (PEI) cellulose layers, with distilled water as eluent [29]. The spots could be visualised by ultraviolet light (254 nm), marked and cut out. Radioactivity was eluted from the PEI cellulose pieces for 1 h with 1 ml 0.1 mol/l HCl/0.2 mol/l KCl at room temperature. Radioactivity was estimated in a liquid scintillation counter, after addition of 9 ml Optisafe 3 and stabilisation for at least 1 h.

dCDA assay

To 100 μl of enzyme-containing supernatant (2–14 μg protein/μl), 20 μl of 5 mmol/l CdR and 80 μl of dCDA buffer were added. This reaction mixture was incubated for 15–45 min at 37°C. The reaction was terminated by adding 50 μl 40% w/v trichloric acetic acid and chilling on ice for 20 min. The denaturated protein was precipitated by centrifugation for 10 min at 10 000 *g*. The supernatant was neutralised with 500 μl trioctylamine: 1,1,2-trichloro-trifluoroethane (1:4). After mixing thoroughly, the mixture was centrifuged for 1 min at 10 000 *g*. The upper layer, containing the nucleosides, was removed carefully. Substrate (CdR) and product (UdR) were separated by reversed phase high performance liquid chromatography (HPLC), using a LiChrosorb 5-RP-18 column with 10 mmol/l ammonium dihydrogen phosphate, pH 6.5 as eluent. Peaks were detected and quantitated using their absorption at 254 and 280 nm.

RESULTS

For each tumour, linearity of deoxycytidine phosphorylation (dCK) and deamination (dCDA) in relation to incubation time and protein content were assessed. For all tumours the dCK reaction was linear until 60 min and the dCDA generally until 45 min, depending on the enzyme dilution. Any disturbance of lysosomal phosphatases or soluble nucleotidases would have been noticed as non-linearity of the assay. Furthermore, dCMP is a very poor substrate for the soluble nucleotidases [30–32], diminishing the chance of interference with the assay. A possible interference with the assay might be the intratumoral concentrations of deoxycytidine triphosphate (dCTP), a feedback inhibitor of dCK. However, cytidine triphosphate (CTP) and uridine triphosphate (UTP) levels as measured in some of our tumours were about 71 and 235 nmol/g, respectively or less (manuscript in preparation), while the dCTP levels in tumour material were about 5 nmol/g (unpublished results). The minimum 8-fold dilution of the tumour sample would prevent any interaction of these nucleotides with the assay. The activities of dCK and dCDA as measured in the linear range are shown in Table 2.

The three colon tumours showed comparable dCK activities; 10.7–12.1 nmol/h/mg protein. The dCK activities in the ovarian tumours were somewhat lower than in the colon tumours, with the lowest activity in Ov.Ri and the highest (9.8 nmol/h/mg protein) in Ov.He In the four sarcomas, dCK activities varied similarly to the ovarian tumours; from 1.8 to 7.4 nmol/h/mg protein. In the 15 squamous cell head and neck tumours dCK activities varied more than in the other tumour types, from 0.4 to 18.4 nmol/h/mg protein.

The dCDA activities varied much more than dCK activities in all tumour types except the soft tissue sarcomas. In this tumour type dCDA activity was remarkably similar and relatively low in all four tumours; from 3.0 to 6.6 nmol/h/mg protein. In the colon tumours dCDA activity varied from 243.3 to 482.7 nmol/h/mg protein. In the ovarian and HNSCC tumours dCDA activity was most varied; from 14.1 to 1230.7 and from 0.6 to 222.0 nmol/h/mg protein, respectively.

The ratios of dCK/dCDA activities were very similar for all three colon tumours (0.025–0.046) and rather similar in the sarcomas (0.581–1.123). In the two other tumour types the differences in dCK/dCDA ratios were larger; 0.004–0.240 and 0.012–4.227 for ovarian and HNSCC, respectively.

The activities of dCK and dCDA have also been determined repeatedly in several tumours obtained at subsequent passages. In the case of some tumour lines (e.g. HNX-DU) these were obtained with an interval of several years. The variation between the passages was not larger than between tumours obtained from the same passage of different mice or even from the same mouse-bearing tumours in both flanks (data not shown). This heterogeneity is probably responsible for the large S.D. found within one tumour type.

In cell lines the pattern of enzyme activity was markedly different from that in the corresponding tumours (Table 3). For example, it was observed that in the four cell lines tested, dCK activity was second highest in the UM-SSC-14C cell line, but of the corresponding tumours, dCK activity was lowest in the HNX-14C tumour. A similar discrepancy was observed for dCDA. There was no consistent difference between cell lines and tumours; based on the activity expressed per mg protein, dCK activity was higher in two (OVCAR-3 and 14C) or four cell lines as compared to the corresponding tumours, but all cell lines showed a lower dCDA activity than the tumours. In the four cell lines dCK activity was higher than the dCDA activity. In the corresponding tumours the reverse pattern was observed.

DISCUSSION

This study demonstrates that dCK activity is widely distributed in solid malignancies, as manifested by its ubiquitous appearance in as many as 28 solid tumours of four different histological origins. The levels of dCK are at least comparable to activities shown in leukaemic cells and normal tissues [3, 10, 13]. Compared to other pyrimidine salvage enzymes, the dCK activities are relatively high [33]. The distribution of dCDA activity shows much more variation both between and within tumours from different histological origins, and are comparable to activities reported by others [10].

dCK plays an important physiological role in the pyrimidine salvage metabolism by catalysing the phosphorylation of CdR to its monophosphate dCMP. dCK is a multisubstrate enzyme; not only does it catalyse CdR phosphorylation, but also that of the purine nucleosides deoxyadenosine and deoxyguanosine. In addition, CdR can also be phosphorylated by other deoxynucleoside kinases such as thymidine kinase (TK2). This broad

Table 2. dCK and dCDA activities in tumours

Tumour	*n*	dCK	dCDA	Ratio dCK/dCDA
Colon				
LS174T	3*,3†	11.3 ± 6.1	245.0 ± 13.1	0.046
SW1398	4, 3	12.1 ± 4.4	482.7 ± 176.1	0.025
WiDr	3, 3	10.7 ± 1.4	243.3 ± 5.7	0.044
Ovarian				
A2780	4, 3	5.8 ± 1.8	28.7 ± 15.9	0.203
Ov. Pe	3, 3	4.6 ± 3.4	1089.7 ± 542.5	0.004
Ov. He	3, 4	9.8 ± 1.0	879.0 ± 99.8	0.011
OVCAR3	3, 3	5.1 ± 0.2	1230.7 ± 82.7	0.004
Ov. Ri (c)	3, 3	3.0 ± 0.9	14.1 ± 1.7	0.212
FKo	4, 2	4.6 ± 2.0	16.5, 22.1‡	0.240
Sarcomas				
S. Ho	3, 3	2.5 ± 0.3	3.8 ± 1.7	0.658
S. Hu	4, 3	1.8 ± 0.9	3.0 ± 1.2	0.581
S. La (c)	5, 3	7.4 ± 3.3	6.6 ± 2.6	1.123
S. To	3, 3	3.1 ± 1.5	4.4 ± 1.1	0.713
HNSSC				
HNX-SK	2, 2	0.4, 4.4‡	42.5, 115.0‡	0.068
HNX-14A	3, 3	9.7 ± 6.7	212.0 ± 30.0	0.046
HNX-14C	3, 4	1.3 ± 0.6	111.0 ± 36.0	0.012
HNX-W	3, 3	14.8 ± 4.8	174.0 ± 90.0	0.085
HNX-LP	4, 3	11.7 ± 7.4	177.0 ± 67.0	0.066
HNX-HE	4, 3	7.8 ± 2.2	18.7 ± 0.6	0.417
HNX-E	3, 3	14.5 ± 5.1	222.0 ± 86.0	0.065
HNX-HN	7, 6	10.5 ± 4.6	35.5 ± 17.0	0.295
HNX-DU	16, 6	18.4 ± 9.4	12.8 ± 7.6	1.438
HNX-OO	2, 2	0.7, 0.8‡	0.6, 1.3‡	0.789
HNX-FR	2, 2	12.8, 12.9‡	171.0, 219.0‡	0.066
HNX-FA	3, 3	15.6 ± 10.6	39.2 ± 20.1	0.398
HNX-22B	4, 3	6.5 ± 3.7	60.7 ± 26.8	0.107
HNX-KE	10, 5	9.3 ± 8.4	< 2.2§	> 4.227
HNX-HEp-2	5, 3	10.8 ± 5.4	9.8 ± 6.9	1.102

Values are means of *n* (*dCK, †dCDA) experiments ± S.D. The activities are expressed as nmol product formed/h/mg protein. Protein content of the tumours was 20–54 mg/g wet weight. ‡Individual values for each measurement of activity. §Of the five measurements of dCDA activity of the HNX-KE tumours, only once was it detected at 2.2, therefore the mean dCDA activity of this tumour is < 2.2.

substrate specificity makes dCK a key enzyme in the DNA precursor synthesis [1–6, 34]. Clinically, dCK is also important. Many leukaemic diseases are treated with deoxynucleoside analogues which are activated by dCK, e.g. ara-C is used in acute myeloid leukaemia [2, 35] and recently fludarabine and especially 2-chloro-deoxyadenosine have been shown to be extremely active against hairy cell leukaemia [36, 37].

The physiological role of dCDA is not completely clear. Its deaminating activity withdraws cytidine and CdR from being phosphorylated by a salvage enzyme, but deamination yields uridine and deoxyuridine, respectively [7, 8], which can be utilised by the salvage pathway enzymes uridine and thymidine kinase. Phosphorylation of uridine ultimately yields UTP, which is an RNA precursor. Phosphorylation of deoxyuridine yields

Table 3. dCK and dCDA activities in cell lines and corresponding tumours

	dCK		dCDA	
	Cell line	Tumour	Cell line	Tumour
WiDr	4.8 ± 1.0	10.7 ± 1.4	1.3 ± 0.8	243.3 ± 5.7
A2780	7.7 ± 3.5	5.8 ± 1.8	0.9 ± 0.6	28.7 ± 15.9
OVCAR3	55.1 ± 9.8	5.1 ± 0.2	9.4 ± 0.2	1230.7 ± 82.7
UM-SCC-14C	47.0 ± 8.2	1.3 ± 0.6	20.7 ± 1.3	111.0 ± 36.0

Values are means of at least three separate experiments ± S.D. The activities are expressed as nmol product formed/h/mg protein. Protein content of the cell lines was 0.1–0.2 mg/10^6 cells.

deoxyuridine monophosphate (dUMP), the substrate for thymidylate synthase, a key enzyme in the synthesis of thymidine nucleotides and an important target in cancer chemotherapy [38]. Thus dCDA may also play a role in the DNA precursor synthesis. Cytidine is the preferred substrate for dCDA, so the role in the synthesis of ribonucleotides may be more important. It has been postulated that dCDA is a downregulated enzyme [39], only active when *de novo* synthesis of deoxyuridine metabolites is not sufficient. The abundant appearance of the deaminated products of ara-C and dFdC in patients [35, 40, 41] indicates that the enzyme is not completely downregulated. However, dCDA activity seems to be not high enough to prevent ara-C or dFdC from exhibiting their antitumour effect, possibly due to a relatively low K_m for phosphorylation as compared to deamination, favouring conversion to the nucleotide [4]. The high activity of ara-C in acute myelogenous leukaemia and of dFdC in non-small cell lung carcinoma [2, 35, 42] supports a sufficient conversion to the nucleotide in the target cells.

One of the reasons for measuring the activities of dCK and dCDA was to determine whether a correlation existed between the enzyme activities and the sensitivity for cytidine analogues. As stated before, the dCK activities in solid tumours are at least as high as the activities in human leukaemic cells and normal tissues [3, 10, 13]. This suggests that these tumours could in principle be sensitive to deoxycytidine analogues. The sensitivity of HNSCC xenografts in nude mice to dFdC and 5-aza-CdR [15, 24], the observed effects of dFdC against ovarian cancer in nude mice [43] and the clinical activity of dFdC against non-small cell lung cancer and ovarian cancer [42], all suggest that the *in vivo* phosphorylating capacity in solid tumours is high enough to convert deoxycytidine analogues to active metabolites. However, a correlation between the level of dCK activity and sensitivity is absent. A panel of HNSCC tumours was tested for the sensitivity to dFdC and 5-aza-CdR [15, 24]. HNX-14C, a tumour very sensitive to both deoxycytidine analogues, has the lowest observed dCK activity, as compared to the other HNSCC tumours tested. There is also no correlation between dCDA activity and sensitivity; of the tumours tested for sensitivity, the highest dCDA activity was measured in HNX-14A, which was very sensitive to dFdC. The dCDA activity measured in the 28 tumours represents the maximum capacity of CdR deamination, which apparently is not fully expressed *in vivo*. The ratio of dCK to dCDA activity is also not correlated with sensitivity. Within the HNSCC panel both the highest and lowest dCK/dCDA ratios were found in the dFdC sensitive tumours as well as for the 5-aza-CdR sensitive tumours [15, 24]. The sensitivity of the cell lines for dFdC [44] also did not correlate. A2780, a very sensitive cell line, has the highest dCK/dCDA ratio (8.5) (Table 3), but other cell lines with comparable sensitivity had varying ratios (from 1 to 5). These findings are in contradiction with those of other authors, who suggested a correlation between dCK activity and sensitivity for ara-C [10, 45, 46]. Our extensive testing may be responsible for that. Our results indicate that, above a certain threshold, dCK activity is not limiting for a tumour or cell line to be sensitive to dFdC and other CdR analogues. This in contrast to the conclusion of Kawasaki *et al.* [47], who postulated that dCK in solid tumours is low compared to lymphoid cells. However, as to solid tumours, this study was limited to immunoprecipitation of dCK in melanoma and astrocytoma only. Our study and that of Madani *et al.* [13] demonstrate a relatively high activity in solid tumours, including those of the brain. Nevertheless, we consider dCK an essential enzyme in the activation of deoxycytidine analogues, since cell lines or tumours with a dCK deficiency are resistant to these drugs [2, 46]. Our results also indicate that dCDA activity, although present, does not necessarily preclude sensitivity of a tumour to a CdR analogue. The differences between the activities of dCK and dCDA in cell lines and their corresponding tumours are striking. Neither an expected similarity between enzyme activities in a tumour and its corresponding cell line was found, nor a constant factor between the dCK and dCDA activities in tumours and cell lines. The difference is probably related to the ideal circumstances under which cells are cultured, in contrast to the growth of tumour cells *in vivo*. Another reason may be the heterogeneity of tumours (necrosis, stroma) as compared to the homogenous cell lines. This indicates that extrapolation of *in vitro* results to *in vivo* should be done with care.

There seems to exist a relationship between the histological origin of the tumour and dCDA activity, especially within the panel of the head and neck squamous cell carcinomas. Of the six oral cavity carcinomas, five have dCDA activities between 111 and 222 with dCK/dCDA ratios under 0.1. Of the hypopharynx carcinomas, four out of five have a relatively low dCDA activity; 0.63–60.7 nmol/h/mg protein, with ratios higher than 0.1. The two laryngeal carcinomas both have a low dCDA activity with a high ratio (> 1). For the other tumour types a smaller number of tumours per classification was available. However, two out of three serous ovarian carcinomas have a low dCDA activity and a dCK/dCDA ratio > 0.2, while both mucinous carcinomas have relatively high dCDA activities with low ratios. The meaning and value of this relationship remains to be investigated.

In conclusion, in 28 solid tumours of various histological origin, dCK activities were relatively high as compared to normal tissues and some leukaemic cell types, as was also observed by Singhal *et al.* [48]. In this latter study dCK activity was also higher in some cell lines than in the corresponding tumour. The levels are within a remarkably narrow range, in contrast to the dCDA activities which showed a 1200-fold variation. No correlation was found between enzyme activities and sensitivity to CdR analogues. A relation between dCDA activity and histological type may be present, i.e. tumours of comparable histological classification showed similar dCDA activities. The antitumour activity observed with cytidine analogues *in vivo* in a number of the tested tumours, demonstrates that the dCK activity is high enough in solid tumours to convert CdR analogues to the active metabolites.

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