Eur J Cancer Clin Oncol, Vol. 24, No. 6, pp. 991–998, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

Sensitivity of Human Neuroblastoma to Activated Dacarbazine: Relationships Between Cell Survival, Methyltransferase Activity and Activation of Adenovirus-5

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Abstract—*Early passage cultures of neuroblastoma cells were tested for (i) cellular sensitivity to the methylating agent* 5-(3-*methyl*-1-*triazeno*)*imidazole*-4-*carboxamide* (*MTIC*); *(ii) ability to reactivate MTIC-damaged adenovirus (Mer$^+$ phenotype); and (iii) methyltransferase activity. Seven of eight lines were resistant to MTIC. One line had an intermediate level of cellular resistance to MTIC, when compared with Mer$^+$ and Mer$^-$ control lines. Methyltransferase activity of the neuroblastomas was intermediate between Mer$^+$ and Mer$^-$ control. Unlike other methylation-resistant cell types, the neuroblastomas showed an initial decline in the MTIC dose–response profile for cell survival followed by a plateau at higher doses. In the virus reactivation assay (HCR), the slope (D_0) of the virus survival curve at high MTIC doses for cells from three of* 10 *patients was similar to that of Mer$^-$ controls. The D_0 for the remaining seven was also much less than for Mer$^+$ controls. However, due to shoulders on the survival curves, all of the neuroblastomas could be classified as Mer$^+$ at low levels of MTIC damage. Overall, the neuroblastoma cells appeared to form a new, though heterogeneous, methylation-resistant group, with cell survival not paralleled by methyltransferase activity or virus reactivation at high methylation levels.*

INTRODUCTION

NEUROBLASTOMA is a common tumor of childhood, with a poor prognosis. There is often an initial response to multidrug therapy, but this is followed by relapse and rapid progression of drug-resistant disease. Therapy protocols usually include DNA alkylating agents (cyclophosphamide, cisplatin, melphalan), vinca alkaloids (vincristine), epipodophyllotoxins (teniposide) and anthracyclines (adriamycin). Dacarbazine [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, DTIC] is an agent which has been shown to have some activity against neuroblastoma in clinical trials and is used as part of multidrug protocols in some centers [1]. Dacarbazine has little activity *in vitro*, but undergoes metabolic activation to MTIC [5-(3-methyl-1-triazeno)imidazole-4-carboxamide], a DNA methylating agent [2, 3]. Sensitivity to MTIC can be attributed to deficient DNA repair [4–6].

Cellular sensitivity to methylating agents has been found to parallel ability to support the growth of methylated adenovirus (Mer$^+$ phenotype; methylation repair proficiency, i.e. Mer$^+$ cells are usually highly resistant to killing by methylating agents compared with Mer$^-$ [7, 8]. Of the many DNA lesions caused by methylating agents, the repair of O^6-methylguanine by a protein with methyltransferase activity is believed to be most closely associated with the Mer$^+$ phenotype [8–12]. Cell lines derived from normal human tissues have the Mer$^+$ phenotype whilst approx. 20% of tumor cell lines and some SV-40-derived lines are Mer$^-$ [7, 8, 12–14].

The fact that neutral tissue has lowered levels of methyltransferase [12, 15] may be relevant to the

Accepted 22 January 1988.
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This work was supported by a grant from the National Health and Medical Research Council, and the Queensland Cancer Fund, Australia.

Abbreviations: DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; MTIC, 5-(3-methyl-1-triazeno)imidazole-4-carboxamide; FCS, fetal calf serum; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methylnitrosourea; PBS, phosphate-buffered saline, pH 7.2; HCR, host cell reactivation; BCNU, bischloroethylnitrosourea.

treatment of neuroblastoma, due to its neural crest origin. It is likely that Mer$^-$ tumors are responsive *in vivo* to alkylating agents such as MTIC (the active product of dacarbazine) and bischloroethylnitrosourea (BCNU) [16].

These considerations make the assumption that the Mer$^-$ phenotype also occurs *in vivo*. This assumption has not yet been adequately tested in humans. In this study, newly established cultures from neuroblastoma patients were screened for sensitivity to MTIC and deficiency of methyltransferase, with a view to detecting Mer$^-$ tumors which may be treatable with DTIC or BCNU. In addition, the ability of cells to reactivate MTIC-treated adenovirus (HCR) was assayed to define the Mer phenotypes of these cells and to determine whether HCR could predict MTIC sensitivity, as found previously in human melanoma cell lines [4].

MATERIALS AND METHODS

Neuroblastoma samples

Neuroblastoma cells were obtained either from tumor biopsies or bone marrow from patients at the Royal Brisbane, Royal Children's and Mater Children's Hospitals, Brisbane. All studies were made on material collected at procedures performed for clinical reasons and remaining after sufficient tissue has been taken for clinical laboratory study.

Solid tumors were dissociated as previously described [17]. Specimens were trimmed of normal tissue, weighed and cut into pieces of less than 1 mm diameter. The mince obtained was centrifuged and resuspended in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal calf serum (FCS) and 0.1% collagenase Type II (Sigma, St. Louis, U.S.A.) to give 50 ml medium/g tissue and then incubated at 37°C. Dissociation was assessed at 4 h and if cell yield was inadequate, incubation was continued overnight. The tissue was then triturated to aid dissociation. Cells were washed twice with medium and counted, using trypan blue dye exclusion to indicate viability. Blood cells were excluded from the counts on the basis of morphology. Heparinized bone marrow specimens were centrifuged once at 150 ***g*** for 10 min and resuspended in RPMI 1640 medium with 10% FCS.

Cell culture

Cell lines were established from clones grown in agar. A 0.55% solution of bactoagar (Difco) in water was boiled for 2 min then cooled to 37% and mixed with an equal volume of hypertonic culture medium [18] that had been sterilized by 0.45 μm membrane filtration. Cells were suspended in the agar medium to give a final concentration of 5×10^3 to 5×10^5 cells/ml for solid tumor specimens and 5×10^4 to 1×10^6 mononuclear cells/ml for bone marrow. Aliquots (1 ml) of this suspension were plated in quadruplicate onto 1 ml 0.5% nutrient agar underlayers in 35 mm Petri dishes. The underlayers, which were used to limit growth of fibroblasts, consisted of bactoagar (final concentration, 0.5%) and hypertonic medium without rat red blood cell lysate. Plates were incubated at 37°C in an environment of 5% O_2, 5% CO_2, 90% N_2, and 100% humidity. Colonies of 40 cells or more were scored at 1–8 weeks. Large colonies were harvested in RPMI 1640 with 10% FCS under sterile conditions and used to establish neuroblastoma cell lines.

Characterization of neuroblastoma cells

The cell lines have been characterized by one or more of the following: (a) noradrenaline secretion, (b) presence of neurosecretory granules by electron microscope (c) positivity for neuron specific enolase (Dako PAP kit, Dako Corp., Santa Barbara, U.S.A.) and (d) recognition by a panel of monoclonal antibodies (A_2B_5, U13A and 127.11) provided by Dr. Kemshead (Imperial Cancer Research Fund Oncology Laboratory, Institute of Child Health, London, U.K.) [19].

Control cell lines

The origins of HeLa-S_3 [20, 21] and the human melanoma MM96 and MM253c1 cell lines have been described [22, 23]. The MM96L line is a late passage subline of MM96. HeLa-S3 and MM253cla have been shown to be Mer$^-$ [4, 20]; MM96L is Mer$^+$ [5].

Cell survival

Cell survival was determined by [^{3}H]thymidine incorporation 5–7 days after treatment. Cultures were seeded in 0.1 ml of medium, in microtiter plates 24 h before treatment with drug (six replicates for each drug concentration) at a cell concentration of 2.5×10^3/ml for control lines and 2.5×10^4/ml for neuroblastoma cell lines. Control lines were seeded at a lower cell concentration because of their faster doubling time. The cell concentration seeded was determined to result in sub-confluent cultures 6–8 days later. Five to 7 days after treatment was initiated the medium was replaced with medium containing [^{3}H-methyl]thymidine (5 μCi/ml, 40 Ci/mmol; RadioChemical Centre, Amersham, U.K.). After 24 h at 37°C the cells were washed with Dulbecco's solution, detached with 1.25 mg/ml pancreatin (Sigma, St. Louis, U.S.A.), 0.16 M NaCl, 8 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4 and 6.0 mM EDTA, pH 7.2, filtered onto glass fiber discs (Whatman GF/C), and lysed and washed exhaustively with water prior to liquid scintillation counting. MTIC was synthesized as previously described [24] and handled under minimal illumination. MTIC

was dissolved in dimethylsulfoxide (100 μl/mg) before dilution with medium at 0°C and further immediate dilution into cultures.

HCR of adenovirus

The procedure was similar to that reported by Parsons and Hayward [25]. Adenovirus was obtained from Dr. A.J. Bellet, John Curtin School of Medical Research, Canberra, Australia, and shown to be mycoplasma-free by agar culture. Virus diluted 1:10 was treated with MTIC in complete medium containing not more than 30% DMSO at 37°C for 30 min in the dark before being diluted further for the replication assays [4].

The ability of cells to reactivate drug-damaged adenovirus (HCR) was assayed by detection of viral antigens 2 days after infection. Successive 4-fold dilutions of virus were used to infect triplicate cultures in microtiter plates (5×10^3 control cells or 5×10^4 neuroblastoma cells/6 mm well) seeded 24 h previously. After 48 h the cells were washed twice with PBS and fixed with methanol for 2 min. The cultures were then incubated for 1 h at 37°C with a 1/20 dilution of human plasma in PBS (20 μl) containing adenovirus antibody followed by peroxidase-conjugated protein A (20 μl of 5 μg/ml; Sigma); each step was preceded by two washes with PBS. After washing with Tris buffer (10 mM, pH 7.4) a mixture of *o*-dianisidine (1 mM) and hydrogen peroxide (2.4 mM) in Tris buffer was added and the reaction stopped after 5–10 min by washing in water. The number of virus-infected cells (stained reddish-brown) in each well were counted and the virus survivals were compared on the basis of D_0, the dose required to reduce survival by a factor of 0.37 on the linear portion of the curve. The limited solubility of MTIC restricted the dose range available for establishing linearity. The slope of the terminal linear portion of the curves was therefore used to calculate D_0. The value of D_q (extent of shoulder) was also calculated for each curve [26].

Methyltransferase activity

The methods were a modification of those used by Margison *et al.* [27]. Cells were suspended in assay buffer (50 mM Tris–HCl, 0.1 mM EDTA, 1 mM dithiothreitol at a concentration of 2×10^7/ml). They were then frozen and thawed, sonicated with 4×10 second pulses and finally the lysate was centrifuged at 20,000 ***g*** for 10 min. The protein concentration of the lysate was then determined by the Coomassie blue method [28]. The substrate DNA containing [^{3}H]O^6-methylguanine prepared by incubating calf thymus DNA with [^{3}H]MNU, was kindly provided by Dr. P. Margison (Department of Carcinogenesis, Paterson Laboratories, Christie Hospital, Manchester, U.K.).

Briefly, total methyltransferase activity was assayed by adding cell lysate (1 mg protein) in assay buffer to [^{3}H]MNU-DNA (specific activity 30 Ci/mmol) containing 0.08 pmol O^6-methylguanine in a total volume of 0.5 ml. Protein concentrations of 0.5–2 mg/ml resulted in a linear relationship between transfer of labelled methyl groups and protein concentration. After 60 min at 37°C, protein and DNA were precipitated by the addition of 170 μl of 4 M perchloric acid. The mixture was heated at 70°C for 30 min, centrifuged and the precipitate washed three times with 4 ml of 1 M perchloric acid. The amount of radioactivity transferred to protein was measured after solubilizing the precipitate in 0.4 ml Soluene 350 (Packard) and 10 ml of toluene scintillant. Counting efficiency was 30%.

Flow cytometry

Cell suspensions (1×10^5 cells/ml) were fixed with 25% (v/v) cold ethanol, resuspended in 200 μl of phosphate buffered saline and then stained with 50 μl of a solution consisting of 1.0% Triton X100, 0.5% RNase and 0.25% propidium iodide. Approximately 1×10^4 cells were analysed at 488 nm for each DNA histogram. The DNA content of the cells was measured using a Becton Dickinson FACS IV. Calculations of percentages of cells in various phases of the cell cycle were made using a curve fitting analysis [29].

RESULTS

Cell survival

Twelve cloned neuroblastoma early passage cell lines (2–6 passages) from 10 patients were used; lines 7-570, 7-609, 11-608 and 11-626 being serial samples from patients 7 and 11 respectively (Table 1). The poor plating efficiency of these cells at very low density in agar or as monolayers prompted the use of a modified clonogenic assay in which clonal growth was compared by [^{3}H]thymidine incorporation 7 days after drug treatment. This method gives similar results to visual counting of colonies [30, 31] and was evaluated further in this study as described below.

Dose–response curves to MTIC were obtained and compared with those from other human tumor lines (Figs 1A and 2A). The profiles of the neuroblastomas tended to show an initial drop followed by a plateau at high doses. The control Mer$^+$ melanoma lines, however, showed an initial shoulder and then fell sharply. This was also the case for other Mer$^+$ lines [6]. There was considerable heterogeneity in response within the neuroblastoma group. Two cultures (1-171 and 13-658) were much more resistant to MTIC than any other cell type previously studied in our laboratories. Even the 9-550 culture (Fig. 2A), which had a level of resistance at low doses intermediate between Mer$^+$ and

Table 1. Cellular response to MTIC, reactivation of damage adenovirus and methyltransferase activity in neuroblastoma, Mer$^+$ and Mer$^-$ cells

Cell line	%S*	MTIC Cell survival AUC† (% MM96L)	HCR D_0 (mM)	HCR D_q (mM)	Viral titer‡(10^6/ml)	Methyltransferase activity (fmol/mg protein)
Neuroblastoma						
1-171	7.5	108.8	16.7	38.1	8.1	113
2-269	3.3		28.6	43.6	9.4	
3-402	4.2	95.4	20.8	20.8	16.5	142
6-530	2.5		38.1	24.4	26.8	
7-570	3.1	98.8	33.9	21.4	19.4	172
7-609	3.1		23.2	36.9	20.6	
8-574	6.7	105.2	26.2	32.1	12.8	
9-550	4.1	70.9	20.8	47.0	39.3	
11-608	2.3	94.8	25.6	28.0	19.8	149
11-626	3.1		32.7	26.8	8.10	
12-622	2.2	91.8	10.7	23.8	9.5	169
13-658	7.3	117.2	10.7	29.8	8.3	68
Others						
MM96L§	9.4	100	57.1	0	33.3	320
HeLa S3‖	7.6	37.4	11.9	0	41.2	43
MM253cla‖	8.5	48.3	14.3	0	21.0	49

*Mean of 2–3 measurements (S.E. < 20%).
†Area under curve expressed as a percentage of the AUC for MM96L (i.e. 0.828 mM).
‡Untreated cells.
§Mer$^+$ control [5].
‖Mer$^-$ control [4, 20].
¶Mean of 3 determinations (S.E. < 10% mean).

Table 2. Proliferative potential of surviving cells 5–7 days post-MTIC treatment

Cell line	Treatment MTIC (mm)	Cell-cycle status* G_1	S	G_2	[^{3}H]Thymidine incorporation/cell (% control)
1-171	Control	64.2	9.1	16.7	—
	0.5	60.5	11.3	19.2	89.5
9-550	Control	84.1	3.6	8.7	—
	0.1	83.9	2.9	8.3	111.5
	0.5	79.6	4.2	10.5	113.3
12-622	Control	93.0	1.6	3.3	—
	0.1	91.7	2.0	3.9	94.1
	0.5	83.3	4.1	9.4	97.2
13-658	Control	87.3	9.7	3.0	—
	0.5	65.5	15.5	8.0	88.2

*% of total population.

Mer$^-$ lines, tended to reach a plateau at 10% survival.

Differences between neuroblastoma lines in cellular sensitivity to MTIC could not be explained by the proportion of cells in S-phase (Table 1). The %S phase figure given in this table represents the proportion of S-phase cells in log-phase cultures prior to seeding. Because of the shape of the survival curves, quantitative comparisons were made on the basis of the area under the curve (Table 1), as used previously for other agents [18, 32].

The [^{3}H]thymidine technique can produce plateau effects in slowly proliferating cells when insufficient time has elapsed for the effects of drug on DNA synthesis to be evident or because cells are merely accumulating in G_2 [31]. To eliminate these possibilities, the DNA histogram of these cells and [^{3}H]thymidine incorporation/cell were measured at the time of measuring thymidine incorporation/well, i.e. 5–7 days after MTIC treatment (Table 2).

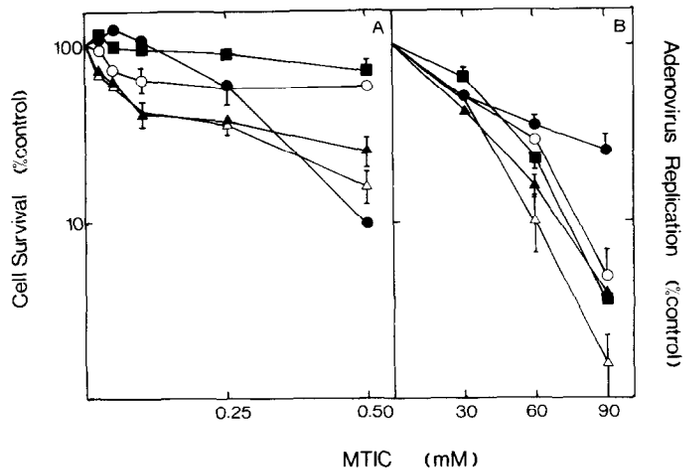

Fig. 1. MTIC dose-response profiles for inactivation of cells (panel A) or adenovirus-5 (panel B). ●, *MM96L (Mer⁺)*, ○, 1-171; ▲, 3-402; ■, 13-658; △, 12-622. *Error bars depict S.E. determined from 3–6 experiments.*

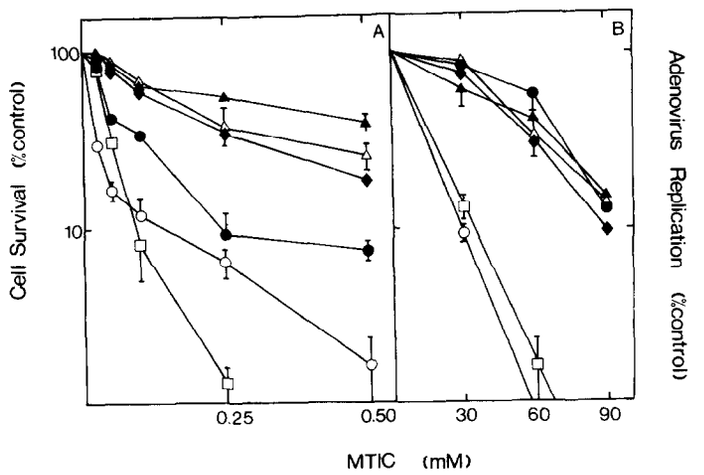

Fig. 2. MTIC dose-response profiles for inactivation of cells (panel A) or adenovirus-5 (panel B). □, *MM253cla (Mer⁻)*; ○, *HeLa-S3 (Mer⁻)*; ▲, 8-574; △, 7-570; ●, 9-550; ♦, 11-608. *Error bars depict S.E. determined from 3–6 experiments.*

There was a small increase in the percentage of cells in G_2 after MTIC treatment in all cell lines. However, the percentage of cells in S-phase was also slightly higher for lines 1-171, 12-622 and 13-658, and unchanged for 9-550. The 1-171, 12-622 and 13-658 cells remaining after MTIC treatment incorporated slightly more [^{3}H]thymidine/cell than the control, untreated neuroblastoma cells, whilst for 9-550 cells [^{3}H]thymidine uptake/cell was slightly decreased. This confirmed that the surviving cells were reproductively active and the plateau is not an artefact of the method.

In separate experiments, lines 1-171, 12-622 and 13-658 cells were pretreated with MTIC, and a survival assay was performed on cells replated 7 days after exposure. There was no difference in sensitivity to MTIC between control and cells pretreated with 0.1 or 0.5 mM MTIC, indicating that the survivors were not a resistant subpopulation (data not shown).

HCR of adenovirus

The neuroblastoma cells were tested for ability to reactivate MTIC-damaged adenovirus, and were compared with Mer$^+$ (MM96L) and Mer$^-$ (MM253c1, HeLa-S3) control lines (Figs. 1B and 2B). The D_0 and D_q values for virus inactivation are given in Table 1. The degree of difference between Mer$^+$ and Mer$^-$ control lines was of similar magnitude to that described by other authors to MNNG [7, 8, 16], i.e. Mer$^+$ D_0 is approx. 3–6-fold higher than Mer$^-$ D_0.

The curves obtained for neuroblastoma cells were characterized by shoulders at low levels of MTIC damage, and thus at low doses the neuroblastoma cells could be classified as Mer$^+$. The typical response in this assay is described by a straight line curve as was the case for the control Mer$^+$ and Mer$^-$ lines. At higher MTIC doses the slopes (D_0) of the neuroblastoma profiles ranged from Mer$^-$ values (1-171, 12-622 and 3-658) to levels inter-

mediate between Mer$^+$ and Mer$^-$.

The serial samples taken from patients 7 and 11 exhibited approximately the same sensitivity as the initial samples. Both patients received chemotherapy in the intervening period with vincristine, cisplatin, teniposide and cyclophosphamide.

Methyltransferase

The activity of methyltransferase in the neuroblastoma cells was measured as transfer of labelled methyl groups from methylated DNA to protein (Table 1). The Mer$^+$ and Mer$^-$ controls differed in activity by a factor of approx. 7×. All of the neuroblastoma cells had lower levels of methyltransferase activity than the Mer$^+$ control. All except for 13-658 were within the range of Mer$^+$ cells described by other authors. The values obtained for Mer$^+$ and Mer$^-$ controls were similar to reported values [12].

The neuroblastomas, which would have been predicted to be Mer$^+$ on the basis of cellular sensitivity to MTIC, also appeared to be Mer$^+$ by methyltransferase assay. However one neuroblastoma with the highest cellular resistance (13-658) had the lowest methyltransferase activity. There appeared to be a relationship between the activity of methyltransferase and the D_0 value obtained in the HCR assay but a satisfactory significance level was not reached (Spearman's rank correlation coefficient, $r_s = 0.6374$, $P > 0.05$, $n = 9$). A lower P value may have been obtained with a larger sample. There was a linear relationship between cell survival and HCR response at the lowest MTIC dose (i.e. 30 mM) ($r_s = 0.6409$, $P < 0.05$, $n = 11$). Neither methyltransferase activity nor HCR D_0 correlated with cell survival ($r_s = 0.1667$, $n = 9$; $r_s = 0.1909$, $n = 11$).

DISCUSSION

The high success rate in this study of obtaining proliferating cultures of neuroblastoma cells provided a unique opportunity for studying methylation resistance in human tumor cells at a much earlier passage level than previous studies. Both clonal assays and measurement of [^{3}H]thymidine incorporation in early passage tumor cells have been reported to reliably predict clinical resistance to drugs (around 90% correct), if the pharmacokinetics of *in vitro* exposure parallel those of *in vivo* administration of the drug [33–35]. With this in mind the present results raise several points of clinical interest. Firstly, treatment of patients with DTIC results in plasma C × T of 50–100 μM.h with a half-life of about 1 h [36]. The pharmacokinetics of MTIC itself are uncertain, but it would appear from the present data that only one of the 10 patients studied (9-550) could possibly have shown a response to a clinical dose of dacarbazine. This is consistent with low response rates previously reported [1, 37]. The fact that the more sensitive 9-550 cells were refractory to high doses of MTIC is consistent with the finding of partial clinical responses. For the purpose of chemosensitivity testing, the 100% success rate in establishing proliferating cultures, including growth from histologically uninvolved marrow (submitted for publication), means that direct determination of cell survival will be a more useful assay than the HCR response.

The present results differ in several respects from the usual pattern of responses of long term human cell lines to methylating agents. Unlike the linear or shoulder plus linear responses obtained previously with MTIC [4, 20], both by us and other authors, the dose responses for survival of neuroblastoma cells generally showed an initial steep slope followed by a less marked response at high doses. This was not due to a kinetically or otherwise resistant subpopulation, or to an artefact of the cell survival method. These results showed that the neuroblastoma as a group were more resistant to high levels of MTIC than other Mer$^+$ cell types; including some of neural crest origin such as melanoma. As stated above these results suggest that dacarbazine would be of limited usefulness in treating neuroblastoma.

Further differences were found when the HCR and methyltransferase levels were determined. The dose responses for HCR in the neuroblastomas were almost the reverse of the cell survival profiles, showing an initial shoulder followed by a sharp drop in virus reactivation (D_0 of 10.7–38.1 mM) which spanned the range between control Mer$^+$ (D_0 57.1 mM) and Mer$^-$ (D_0 11.9, 14.3 mM) cell lines. The HCR results at low doses correlate with cellular resistance to MTIC, as is the case in melanoma cells [5], but the observed difference from Mer$^-$ controls is not as great as that indicated by cell survival assay.

Measurement of methyltransferase activity gave the expected result for the Mer$^+$ and Mer$^-$ control lines (320, 43, 49 fmol/protein respectively); with the Mer$^+$ line having approx. 7 times the activity of Mer$^-$ lines. In the neuroblastoma group, cell survival and methyltransferase activity (113–172 fmol/protein) both indicated the cells to be Mer$^+$; (with the exception of 13-658, 68 fmol/mg protein). The methyltransferase levels were all however lower than the Mer$^+$ control. The ability to reactivate MTIC-treated virus gave conflicting results. Neuroblastoma cells appeared to be Mer$^+$ at low levels of damage, but Mer$^-$ at higher levels of damage. Methyltransferase activity appeared to be related to HCR response at high dose. The results raise the possibility that DNA repair (ostensible O^6-methylguanine repair) as judged by HCR and methyltransferase activity is not a significant factor

in the high level of cellular resistance to MTIC toxicity.

O^6-Methylguanine is principally a promutagenic lesion in bacteria, having little cytotoxic activity [12]. Lack of methyltransferase activity in mammalian cells has been found to be highly correlated with cellular sensitivity to methylating agents [9–12] but there is strong evidence to suggest that O^6-methylguanine is not a cytotoxic lesion [38]. For example, Karran and Williams [39] showed that depleting methyltransferase activity by treating Raji lymphoma cells with O^6-methylguanine did not sensitize them to killing by MNNG. Several groups have described cell lines which are resistant to MNNG but have low to negligible methyltransferase activity [20, 40, 41].

It seems that methyltransferase activity in established cell lines is usually paralleled by an activity capable of protecting cells from the cytotoxic effects of alkylating agents. MTIC resistance in neuroblastoma cells, however, appears to be only partially linked to methyltransferase activity and the HCR response. In the latter assay, the high frequency of base methylation required to inactivate adenovirus may invoke a different type of repair than the alkylation levels achieved in cellular DNA. This is consistent with the finding tht HCR response at low, but not high, levels of damage paralleled cellular sensitivity.

While the mechanism(s) of cellular resistance are not yet clear, it is possible that the neuroblastoma responses are due to a combination of factors such as early passage, derivation from low methyltransferase neuronal cells, and neoplastic transformation. The plateau-type cell survival profiles suggest that it will be of interest to compare drug transport, repair of lesions other than O^6-methylguanine and cell cycle kinetics in these and other cell types.

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