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Chemosensitivity in childhood brain tumours *in vitro*: evidence of differential sensitivity to lomustine (CCNU) and vincristine

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Abstract

The aim of this study was to examine the range of sensitivity of a panel of short-term cultures derived from different types of malignant childhood brain tumours including medulloblastoma, ependymoma and glioblastoma multiforme to three cytotoxic drugs, lomustine (CCNU), vincristine (VCR) and procarbazine (PCB). Sensitivity was assessed using a modification of the dimethylthiazolyl-2,5-diphenyl tetrazolium bromide (MTT) assay. Short-term cell lines derived from ependymomas were considerably more resistant to VCR than other types of childhood brain tumours, while cultures derived from supratentorial primitive neuroectodermal tumour (PNET) displayed marked sensitivity to both lomustine and VCR. Cultures from ependymomas, medulloblastoma and astrocytic gliomas had similar sensitivity to lomustine and PCB as cultures derived from adult malignant astrocytoma.

Keywords: Medulloblastoma; Astrocytoma; Ependymoma; Central neuroblastoma; Dimethylthiazolyl-2,5-diphenyl tetrazolium bromide (MTT) assay; Chemosensitivity; Cell culture; Brain tumour

1. Introduction

One of the outstanding successes of cancer treatment over the last 20 years has been the role of adjuvant chemotherapy in improving the survival of children with cancer. Over two-thirds of children with acute lymphoblastic leukaemia, Hodgkin's disease, non-Hodgkin's lymphoma and Wilms' tumour are now cured following adjuvant chemotherapy [1]. Brain tumours account for 20% of all cancers in children [2,3] and are the second most frequent cause of death in children under 15 years of age [3]. However, although the mortality of children with cancer has been declining steadily over the last two decades, this decline has been less marked in children with brain tumours [4]. Although a high proportion of some types of paediatric brain tumour like pilocytic astrocytoma can be cured surgically and nearly 60% of patients with medulloblastoma are cured, the malignant astrocytomas of childhood, ependymoma and a significant proportion of medulloblastomas have proved largely refractory to treatment. There is a pressing need for the development of effective chemotherapy regimens for these tumours. Not only would this result in longer survival, it would also reduce the impact of radiation-induced toxicity [5,6], by using cytotoxic drugs to replace, reduce the dose or delay radiation treatment without compromising long-term survival. However, the conventional approaches of drug development through large-scale multi-institutional trials have been slow. It would therefore be of considerable interest to be able to use *in vitro* model systems to screen drugs for efficacy against a range of different childhood brain tumours. The purpose of this study was 2-fold; firstly, to determine the degree of heterogeneity in response to cytotoxic drugs used in the treatment of children with brain tumours and secondly, to determine if there are systematic differences in chemosensitivity between different groups of childhood brain tumours and to compare this with data derived from adult patients with glioblastoma multiforme.

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2. Patients and methods

2.1. Biopsy samples

Biopsy samples were received from a total of 33 children or young adults who underwent neurosurgical resections at the National Hospital for Neurology and Neurosurgery, Queen Square, London, The Great Ormond Street Hospital for Children, Great Ormond Street, London, UK and from the Queen's Medical Centre, Nottingham, UK. The definitive diagnosis of the samples was provided following review of the original case material by a consultant neuropathologist at each hospital/centre using the current World Health Organization (WHO) classification system [7]. The clinical characteristics of these patients are summarised in Table 1. In addition to the short-term cultures derived directly from patients, one established cell line, DAOY, derived from a medulloblastoma [8] was included in the study.

2.2. Cell culture

Biopsy samples were immediately placed in biopsy collection medium (Ham's F-10 medium, buffered with 25 mM Hepes (Gibco BRL, Paisley, UK) containing 10% (v/v) selected fetal calf serum (FCS) and 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin and 20 μg/ml amphotericin B (Fungizone, ICN Biomedical) and transported to the laboratory at ambient temperature. Non-tumour material was removed by dissection and the tumour chopped into fragments using a new set of scalpel blades for each biopsy in order to minimise mechanical damage to cells. The tumour fragments were collected and resuspended in biopsy collection medium. Once the large fragments had settled, the supernatant containing necrotic material was removed and after a further cycle of washing, the tumour fragments were resuspended in complete growth medium (Ham's F-10, buffered with 25 mM Hepes and supplemented with 10% (v/v) selected FCS and 50 units/ml penicillin and 50 μg/ml streptomycin) and transferred to a 25 cm^2 cell culture flask (Nunclon, Gibco). Sufficient collagenase solution (Sigma grade 1A collagenase dissolved in Hanks' balanced salts solution (HBSS) at a concentration of 2000 units/ml) was added to give a final working concentration of 200 units/ml. After 18–24 h incubation at 37°C, the fragments were disaggregated by gentle pipetting, pelleted by centrifugation at 100*g* and the cells were resuspended in fresh complete growth medium, counted and transferred to a new cell culture flask. After 18–24 h incubation at 37°C, the medium and any non-adherent material were removed and the culture re-fed. All cultures were found to be negative for contamination by mycoplasma using a modified fluorochrome method [9].

2.3. Chemosensitivity assay

Cultures in the exponential growth phase were trypsinised and diluted in complete growth medium to give a total cell count of between 1 and 2×10^4 cells/ml. One hundred microlitres of cell suspension was added to each well of a 96-well flat-bottomed microtitration plate (Nunclon, Gibco). The plates were then sealed with a non-toxic Mylar sealer (Titertek, ICN Biomedical) and incubated at 37°C for 72 h to ensure that the cells were in exponential growth. To ensure that the cells remained in exponential growth throughout the assay, a separate plate was prepared for each cell line and at daily

Table 1
Samples examined in the study ($n = 33$)

Tumour	Sex	Number of patients	Site	Age range (years)	Primary or recurrent?
Medulloblastoma	Male	10	Posterior fossa	3–15	9 primary 1 recurrent
	Female	3	Posterior fossa	3 months–10	3 primary
Ependymoma	Male	6	4 Posterior fossa 1 4th ventricle 1 Hypothalamus	1–12	5 primary 1 recurrent
	Female	1	Posterior fossa	8	1 primary
Pilocytic astrocytoma	Male	3	Posterior fossa	5–19	3 primary
	Female	3	Posterior fossa	2–4	3 primary
Glioblastoma multiforme	Male	1	Posterior fossa/brain stem	6	1 primary
	Female	4	1 Left occipito-parietal 1 Left frontal 1 Left parietal 1 Pineal gland	9–17	3 primary 1 recurrent
Supratentorial PNET	Male	2	1 Occipito-parietal 1 Posterior fossa	5–11	2 primary
	Female	0			

PNET, primitive neuroectodermal tumour.

intervals, medium was aspirated from six wells, the cells were washed twice with 100 μl HBSS and once with 100 μl of trypsin solution (0.25% w/v, Gibco). The cells were then incubated with 0.1 ml fresh trypsin, resuspended in Isoton II and counted in a precalibrated Model ZM Coulter Counter (Coulter Electronics, Luton, UK).

The concentration ranges were chosen to bracket the concentrations which might be achieved clinically in patients with brain tumours. These concentration ranges were as follows: procarbazine (PCB) 4–5000 μg/ml, lomustine (CCNU) 0.3–50 μg/ml and vincristine (VCR) 3×10^{-5}–10 μg/ml. PCB and VCR were dissolved in Ham's F10 medium (without FCS or antibiotics) and lomustine was dissolved in absolute ethanol. Aliquots of stock solutions sufficient for individual assays were stored at −70°C. Drug breakdown was not determined, but assumed to be minimal at this temperature. For each experiment, drug solutions were freshly prepared in complete growth medium from the stock solutions. The concentration of ethanol present in the highest drug concentration used in this study was non-toxic to malignant glioma cells *in vitro* (data not shown).

Once the cells were in exponential growth, the growth medium was removed and replaced with 100 μl of the appropriate drug dilution. Two wells in each column were re-fed with complete growth medium to act as controls. Each plate was then re-sealed with a plate sealer and incubated at 37°C. Drug solutions were renewed at 24 and 48 hourly intervals, resulting in cells being exposed to the drugs for a total of 72 h. Following drug exposure, the cells were washed twice with 100 μl of HBSS to remove any residual drug and then re-fed with 100 μl of fresh complete growth medium and incubated for a 48-h recovery period with the growth medium being replaced at 24 h. Following recovery, the growth medium was removed and 100 μl of dimethyl thiazolyl-2,5-diphenyl tetrazolium bromide (MTT) solution was added to each well. To minimise spontaneous formazan production, MTT (Sigma) was made up in Ham's F-10 without FCS at a concentration of 1 mg/ml and filter-sterilised using a 0.22 μm filter. The cells were incubated for 4 h and the supernatant carefully aspirated to avoid disturbing any formazan crystals. One hundred microlitres of DMSO (Sigma) was added to each well and the plates were shaken gently for 2–3 min on a plate shaker to ensure complete solubilisation of the formazan crystals. The absorbance of each well was then determined using a Dynatech MR600 plate-reader at a wavelength of 570 nm. The reader was blanked against wells that contained medium and MTT, but no cells. The absorbance values were then transferred to a spreadsheet programme, which produced a dose–response curve and calculated the ID_{50} (the dose of drug that inhibited MTT–formazan production by 50%).

2.4. Statistical analysis

The Mann–Whitney test was used to compare the ID_{50} of the different drug treatments for each type of cell line. A P value of <0.05 was taken as the level of significance.

3. Results

Typical dose–response curves for each of the drugs used in the study are given in Fig. 1. Each culture was assayed within 1 to 3 passage levels on between three and nine occasions (Fig. 1).

3.1. Lomustine

Cultures derived from medulloblastoma displayed a 4.7-fold variation in ID_{50}s ranging between 2.8 and 13.1 μg/ml with a median ID_{50} of 6.8 μg/ml (Fig. 2a). Cultures derived from ependymomas displayed a similar range of sensitivity with ID_{50} values between 3.2 and 10.1 μg/ml and a median of 6.3 μg/ml. Cultures derived from astrocytic gliomas were slightly more resistant to lomustine and the range of ID_{50}s for these tumours was less broad than for short-term cultures derived from medulloblastomas or ependymomas. The ID_{50} values for glioblastoma multiforme cultures lay in the range of 7.9–10.4 μg/ml, with a median value of 8.2 μg/ml while the ID_{50} values for pilocytic astrocytomas were in the range of 5.5–11.6 μg/ml with a median value of 8.15 μg/ml. The slight resistance of the astrocytomas compared with the ependymomas and medulloblastomas did not reach statistical significance. Strikingly, both cultures derived from supratentorial primitive neuroectodermal tumour (PNET) appeared to be markedly sensitive to lomustine and significantly more sensitive to this drug than cultures derived from medulloblastoma (Mann–Whitney test, $P=0.044$) and pilocytic astrocytomas ($P=0.036$). Both cultures derived from supratentorial PNETs were more sensitive to lomustine than cultures derived from glioblastoma and ependymoma, but this did not reach statistical significance ($P=0.067$ and $P=0.071$, respectively). DAOY had an ID_{50} of 8.2 μg/ml and therefore fell towards the more resistant end of the range of sensitivities recorded for the short-term cultures derived from medulloblastoma.

3.2. Procarbazine (PCB)

Ependymomas and pilocytic astrocytomas had similar and rather narrow ranges in ID_{50} when treated with this drug (Fig. 2b). Ependymomas had ID_{50}s in the range of 1347–3022 μg/ml (median 2586 μg/ml), a 2.2-fold range, whilst pilocytic astrocytomas had ID_{50}s between 1987 and 3628 μg/ml (median 2573 μg/ml), a 1.8-fold range. Cultures derived from glioblastoma multiforme and

medulloblastomas had more variation in their sensitivity to PCB with some cultures displaying comparative sensitivity to the drug. For the glioblastomas, the ID_{50}s ranged between 469 and 7157 μg/ml (median 2087 μg/ml), a 15.3-fold range, while medulloblastomas had ID_{50}s between 905 and 17 070 μg/ml (median 2548 μg/ml), an 18.9-fold range. In contrast, one culture derived from a medulloblastoma and one from a glioblastoma was markedly resistant to PCB *in vitro*. There was no evidence of differential sensitivity of the cultures derived from supratentorial PNETs to this drug and DAOY was relatively resistant to procarbazine (ID_{50} 5083 μg/ml).

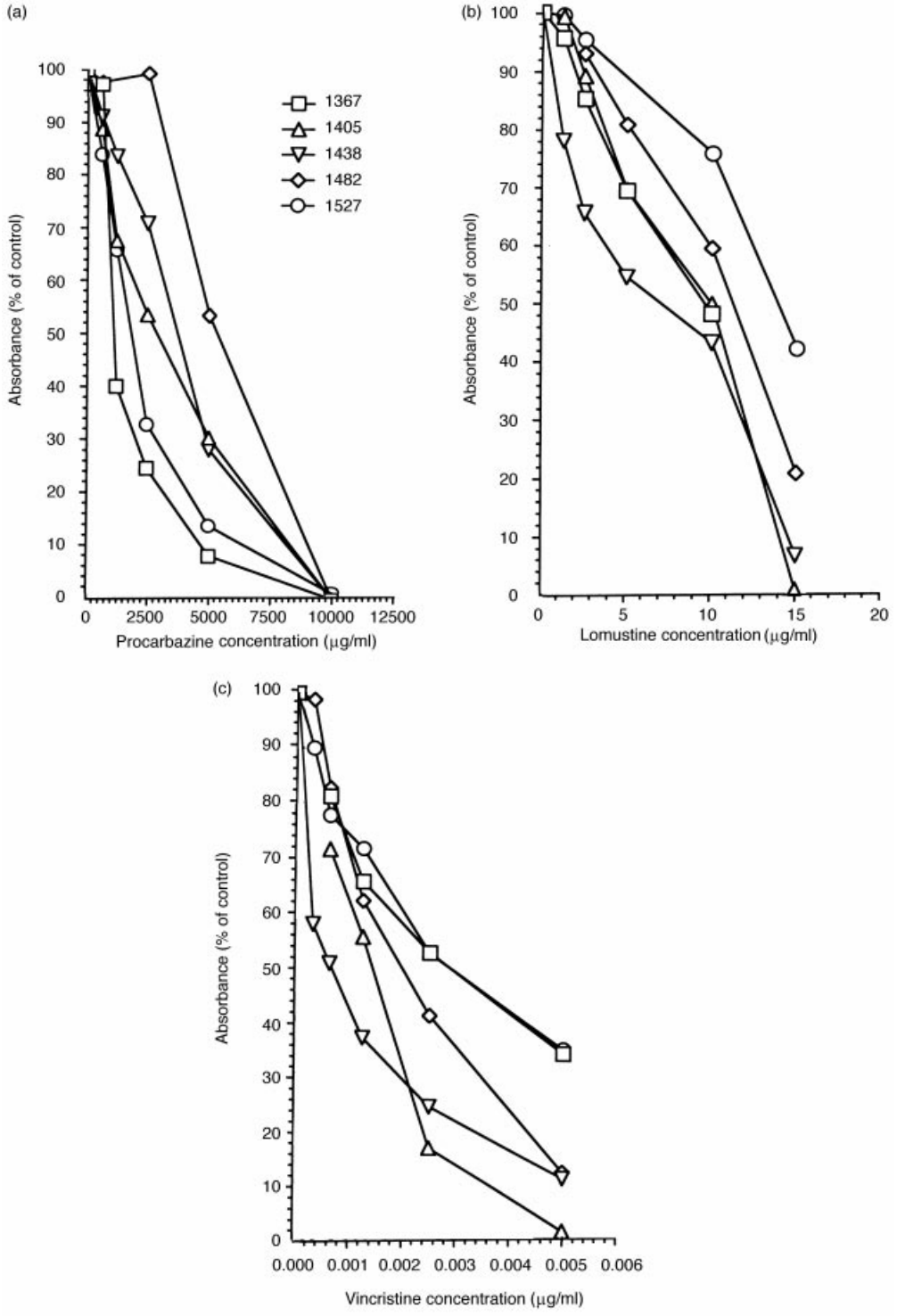

Fig. 1. Typical dose–response curves for five short-term medulloblastoma cell lines treated with (a) procarbazine (PCB); (b) lomustine and (c) vincristine (VCR). These curves are the result of a number of replications (IN 1367 $n=3$ IN 1405 $n=5$, IN 1438 $n=5$, IN 1482 $n=7$, IN 1527 $n=9$).

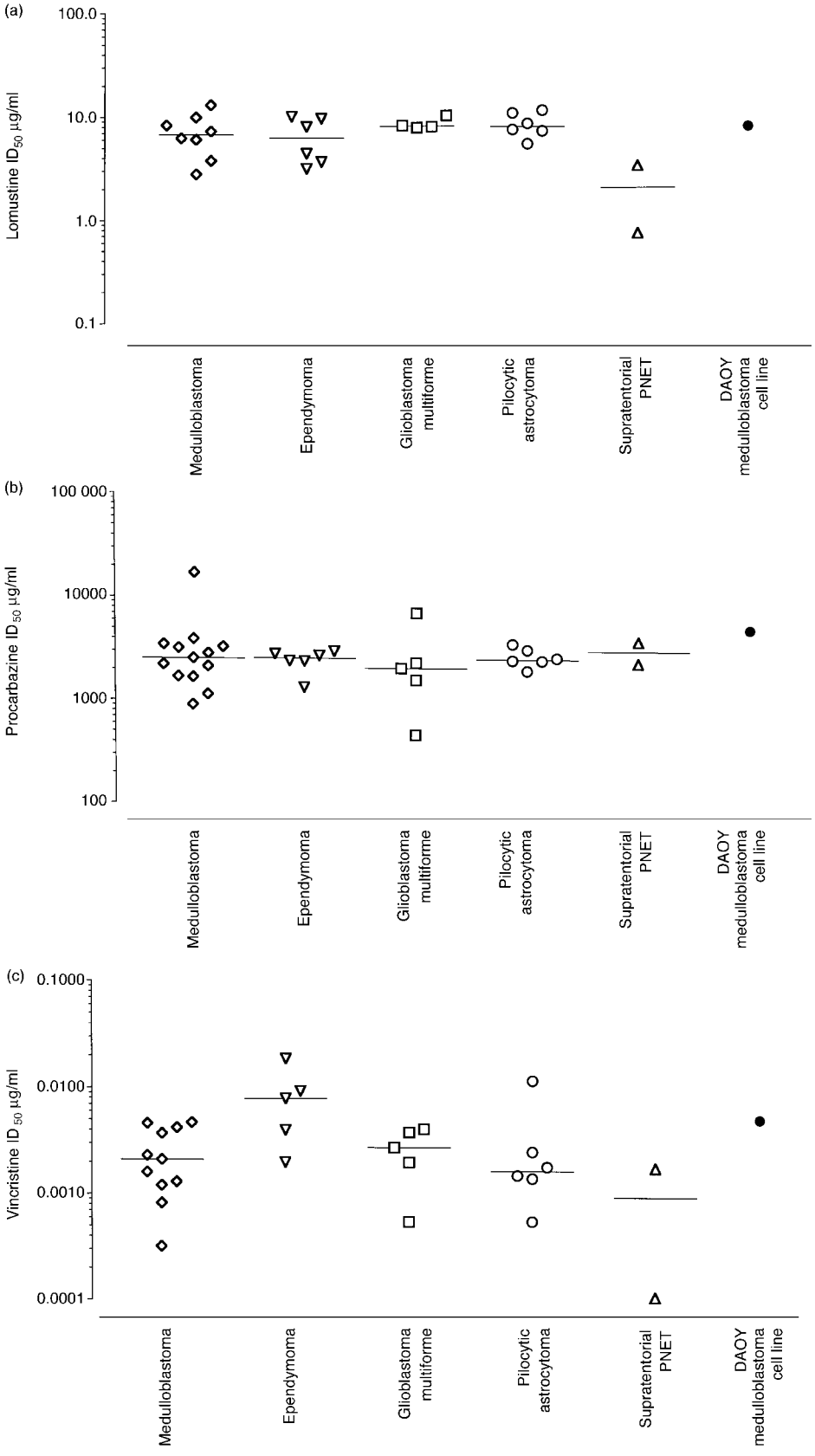

Fig. 2. Scatterplot of ID_{50} values for panels of short-term cultures derived from medulloblastoma, ependymoma, glioblastoma multiforme, pilocytic astrocytoma, supratentorial primitive neuroectodermal tumour (PNET) and the medulloblastoma cell line, DAOY to (a) lomustine (CCNU), (b) procarbazine (PCB) and (c) vincristine (VCR). Horizontal line indicates the median ID_{50}.

3.3. Vincristine (VCR)

The distribution of ID_{50}s for VCR was considerably wider than that of either lomustine or PCB (Fig. 2c). Cultures derived from medulloblastoma had ID_{50}s in the range of 3.2×10^{-4}–4.7×10^{-3} μg/ml (a 15-fold difference, median 2.1×10^{-3} μg/ml), glioblastomas a range of 5.5×10^{-4}–4.1×10^{-3} μg/ml (a 7-fold difference, median 2.8×10^{-3} μg/ml) and pilocytic astrocytomas a range of 5.5×10^{-4}–1.2×10^{-2} μg/ml, (a 22-fold difference, median 1.7×10^{-3} μg/ml). Short-term cultures derived from ependymoma were markedly resistant to VCR with ID_{50} ranging from 2.0×10^{-3}–1.9×10^{-2} μg/ml (median 8×10^{-3} μg/ml), while cultures derived from supratentorial PNETs appeared to be generally sensitive to this drug with ID_{50} values of 1.1×10^{-4} and 1.7×10^{-3} μg/ml. Cultures derived from ependymomas were significantly more resistant to VCR than cultures derived from medulloblastomas (Mann–Whitney test, $P=0.021$), pilocytic astrocytomas ($P=0.041$), glioblastomas ($P=0.048$) and supratentorial PNETs ($P=0.048$).

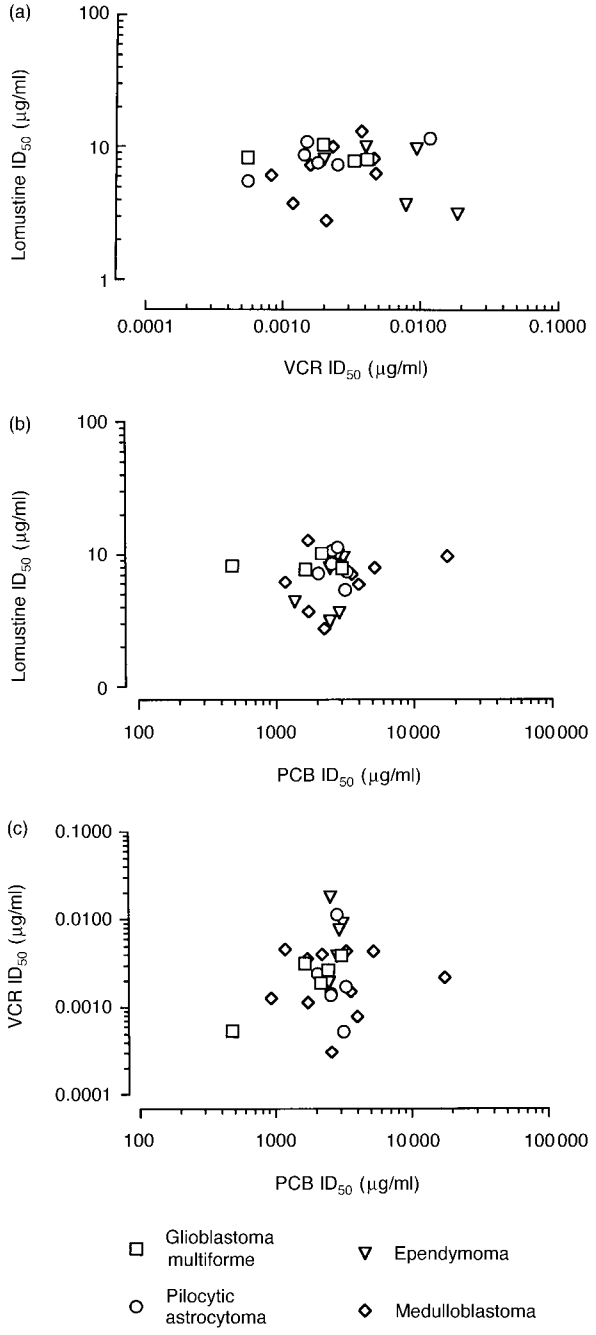

Fig. 3. Patterns of cross-resistance for four types of childhood brain tumours in short-term culture: (a) lomustine (CCNU) versus vincristine (VCR); (b) procarbazine (PCB) versus lomustine; and (c) PCB versus VCR.

3.4. Patterns of cross-resistance

In order to determine if there was any evidence of cross-resistance between any of the drugs used in the study in any of the different types of childhood brain tumours, the ID_{50} values were compared for each group using a regression analysis. In no group of tumour cell lines was there any evidence of cross-resistance between any of the drugs (Fig. 3a–c), although one culture derived from a glioblastoma appeared to be markedly sensitive to both PCB and VCR. However, another culture, derived from a medulloblastoma that was extremely resistant to PCB did not show collateral resistance to VCR.

3.5. Relationship between chemosensitivity and culture doubling time

There was no relationship between sensitivity to any of the three drugs tested and culture doubling time (data not shown).

4. Discussion

The use of short-term cell cultures derived from human malignant astrocytomas in adults has provided a good model system to investigate the biological and therapeutic properties of these tumours. It has, however, been more difficult to produce reliable *in vitro* model systems for brain tumours in children, the greatest difficulty being the inability to establish cell lines in significant numbers from any type of childhood brain tumour. A limited number of cell lines have been produced from medulloblastoma, which fall phenotypically into two categories. The first grow as a suspension of single cells or loosely attached aggregates of cells displaying little or no adherence to the growth substrate [10–14] while the second group of cultures, which includes DAOY, grow as adherent monolayers [15–19]. Both types of cell line display similar immunophenotypic and genotypic characteristics. There are only a

handful of reports describing the establishment of cell lines from ependymomas and none of these cell lines appears to be fully characterised or widely available [20] and there are no reports of established cell lines derived from paediatric astrocytic gliomas.

This means that low-passage short-term cell lines are likely to be an important source of material for biological study, but how representative are these cultures of the tumours of origin? The cell lines in the present study grew as adherent monolayers with no tendency to grow in suspension. We have been able to show that these cultures display immunophenotypic characteristics consistent with the tumour of origin. Short-term cultures derived from medulloblastoma do not express GFAP, but some do express 'mature' neuronal features like the 160 kDa neurofilament protein, which is the predominant isoform expressed in these tumours *in situ* and in other established cell lines [21,22], and virtually all express synaptophysin, an 'immature' neuronal feature. Cultures derived from ependymomas do not express neurofilament protein, but do sometimes express GFAP, especially when grown on surfaces coated with basement membrane components. Cultures derived from pilocytic astrocytomas commonly expressed GFAP while cultures derived from malignant astrocytomas also expressed this antigen, but less commonly than the low-grade astrocytomas. A detailed immunophenotypic characterisation of these cell lines described in this study has been carried out of which a report is in preparation (data not shown).

G-banded and molecular cytogenetic studies reported in the literature, as well as our own studies carried out using the cell lines described in this paper, and others derived in the same way provide convincing evidence that these cultures are composed of neoplastic cells. In short-term cultures derived from ependymomas, monosomy of 22 was common and structural abnormalities of this chromosome were frequently observed, as were abnormalities involving chromosomes 1, 6 and 17 and numerical abnormalities involving chromosomes 7, 9 and 12 [23,24]. Similarly, it has been possible to detect karyotypic abnormalities in short-term cultures derived from medulloblastoma which include translocations involving chromosomes 1, 9, 17 and 22 [25–27]. Paediatric astrocytomas appear to give rise to cell lines that have comparatively simple karyotypic changes although subsets of cells appeared to have structural abnormalities involving chromosomes 3, 7q, 9q or 17p [27]. Recently, we have extended our genetic characterisation of these and other short-term cell lines using comparative genomic hybridisation and it has been possible to show that there are often large numbers of numerical aberrations in short-term cultures produced from ependymomas, medulloblastomas and paediatric malignant astrocytomas which have not been detected by classical cytogenetic analysis (data not shown).

The chemosensitivity assay used in the present study has been extensively evaluated and it is clear that the use of long-term drug exposure (>1 population doubling time) followed by a recovery period of similar length yields cytotoxicity data which is comparable with that produced by a monolayer or soft-agar clonogenic assay [31]. This correlation is independent of the final endpoint used, whether it be isotope uptake measured by scintillation spectrometry or scintillation autofluorography [31], MTT dye reduction [32] or sulphorhodamine staining [33], all produce essentially identical dose–response curves.

With any chemosensitivity assay, it is of critical importance to ensure that responses of target cells occur at concentrations that might reasonably be achieved *in situ*. The levels of lomustine that can be achieved clinically in adults with malignant cerebral glioma are in the range of 5–10 μg/ml [34]. Although there are no systematic data on the penetration of this drug into paediatric brain tumours, taking 5 μg/ml as a cutoff concentration, only 3/6 (50%) medulloblastoma, 1/6 (17%) ependymoma, 2/8 (25%) pilocytic astrocytoma and none of the glioblastoma had ID_{50}s which lay below this concentration. This suggests the potential of only modest clinical sensitivity for this drug against these tumours. Both supratentorial PNETs had ID_{50}s below the clinically achievable drug level suggesting the possibility of clinical sensitivity. Information about the penetration of *Vinca* alkaloids into human brain tumours is extremely limited. A single study of a patient with an intracranial epithelioid sarcoma who received 200 μCi of radio-labelled vinblastine 4 weeks before she died [35] reported levels as high as 68 ng/g in the necrotic core of the tumour, while in normal brain, 4 cm from the tumour, the concentration of drug was 5 ng/g. At this concentration of vincristine only 1/11 cultures derived from medulloblastoma (9%), 1/5 glioblastoma (20%), 1/6 low grade astrocytoma (17%) and 1 culture (50%) derived from a supratentorial PNET would be sensitive to this drug. No cultures derived from ependymomas had ID_{50}s below this drug concentration, suggesting that these tumours are unlikely to respond to this drug clinically. There are no data available about the penetration of PCB into intracranial tissue, making a comparison between *in vitro* sensitivity and *in situ* drug levels impossible.

What evidence is there that any of the childhood brain tumours included in the present study are clinically sensitive to PCB, lomustine or VCR? Ependymomas are clinically unpredictable. They may be histologically 'well differentiated' or 'anaplastic' although in practice histological appearance does not appear to predict long-term outcome. Small group studies of combination chemotherapy with vincristine, procarbazine, prednisone (MOPP) (which contains both PCB and VCR) as the primary post-surgical treatment show this is as

effective as radiotherapy in producing long-term survival (and with less intellectual impairment) [36]. However, in larger studies, the only clinical parameters which appear to predict outcome are extent of surgical resection [37,38] and the amount of residual tumour on postoperative imaging [38]. Chemotherapy with either lomustine, VCR and prednisone [39] or with the "eight drugs in one day" regimen [40] did not influence outcome in these tumours suggesting that they are intrinsically resistant to a wide range of cytotoxic drugs. The *in vitro* data presented here is consistent with the clinical data. There is little evidence of sensitivity to either lomustine or PCB and there is evidence for a marked resistance to VCR in most cell lines *in vitro*. This is consistent with studies that have shown that these tumours express P-glycoprotein *in situ* [41,42]. This is likely to be relevant to the design of adjuvant chemotherapy regimens for these tumours that include drugs, which are substrates for P-glycoprotein. It has been suggested that the addition of adjuvant chemotherapy comprising lomustine, VCR and prednisone to radiation following surgery is of benefit in children with malignant astrocytomas [43], and this seems to hold true in children whose tumours have been totally resected. Although in the present study there is no evidence of a greater sensitivity of glioblastomas versus other brain tumours to lomustine *in vitro*, they do appear to be more sensitive to VCR than ependymomas and one culture showed marked sensitivity to PCB. There is no evidence *in vitro* for differential sensitivity of pilocytic astrocytomas to any of the drugs tested, although there was a slight scattering following VCR treatment, and this is consistent with the limited clinical data regarding the effectiveness of chemotherapy in children with unresectable, progressive low-grade astrocytoma [44]. Medulloblastoma is usually treated with maximal surgical resection followed by craniospinal irradiation. There are some historical data that suggest that lomustine and PCB [45], as well as VCR [46] can produce responses in a proportion of patients with medulloblastoma. The efficacy of adjuvant chemotherapy of medulloblastomas with lomustine and VCR has been assessed in two major trials [47,48] which indicated that while not all patients derived benefit from chemotherapy, some subgroups including those with extensive disease, partial resection or those with brain stem involvement did. More recently, the introduction of adjuvant chemotherapy with lomustine, VCR and cisplatin has improved disease-free survival in both standard-risk and high-risk patients with this tumour, even if the dose of craniospinal irradiation is reduced [49,50]. This suggests medulloblastomas are sensitive to chemotherapy and there is certainly evidence in the present study for sensitivity to VCR in a proportion of cases, but not to PCB or lomustine. It is noteworthy that DAOY does not appear to have a sensitivity profile typical of the short-term cultures derived from medulloblastoma, it is resistant to the drugs used in the study and resembles more closely the sensitivity of cultures derived from astrocytic gliomas.

The response of short-term cultures derived from medulloblastomas produced in a similar manner to those in the present study have been reported previously [51]. Tomlinson and colleagues found, using an isotope uptake assay, that 7/12 (58%) such tumours were sensitive to carmustine (BCNU) at clinically achievable doses (mean and median ID_{37} was 3.3 μg/ml and 2.0 μg/ml, respectively). In the present study, medulloblastoma cell lines were somewhat more resistant to lomustine with ID_{50}s in the range 2.8–13.1 μg/ml, with a median value 6.8 μg/ml. It is not clear if this due to the greater cytotoxicity produced by carmustine *in vitro*, although this is unlikely in view of the data from adult astrocytomas discussed below. When the same 13 cultures were assayed for sensitivity to vincristine it was clear that the cultures in the present study were considerably more sensitive to this drug than the cultures reported previously. This is almost certainly because of the very short exposure time (1 h) used. In the present study, the medulloblastoma cell lines were found to be quite sensitive to VCR with a mean ID_{50} of 0.0024 μg/ml. Interestingly, the established medulloblastoma cell line, DAOY had a mean ID_{50} of 0.005 μg/ml, nearly double that of the short-term cell lines.

Is there any evidence that childhood brain tumours are different in their chemosensitivities to adult astrocytomas? In the present study, the sensitivity of cultures derived from paediatric astrocytomas were very similar to those reported for adult astrocytomas treated *in vitro* with either carmustine [52] or lomustine [53]. Using a cell counting assay, Kornblith and colleagues found that cultures derived from low grade astrocytoma had ID_{50}s in the range 3.9–22.4 μg/ml and cultures derived from high-grade tumours were in the range 3.4–23.8 μg/ml (median 7.9 μg/ml). In a larger series, Thomas and coworkers found that the median ID_{50} of cultures derived from malignant astrocytoma was 9.5 μg/ml [53]. These data are very similar to the present series where the median ID_{50} value was 8.15 μg/ml for pilocytic astrocytomas and 8.2 μg/ml for the high-grade glioblastoma multiforme cultures.

Studies of testing brain tumours in children for *in vitro* chemosensitivity have not been widely carried out despite the clinical evidence that some of these tumours are likely to be more responsive to chemotherapy than their adult counterparts. There are, however, significant clinical advantages that might accrue from a systematic investigation of the *in vitro* sensitivity of these tumours. Screening short-term cultures derived from different types of brain tumours may help to speed up the process of drug development by helping to identify promising new compounds in "*in vitro* phase II trials". However,

the major aim must be individualised chemosensitivity testing to determine within a single type of tumour which patients will respond. This is likely to be especially important in younger children where effective chemotherapy would reduce the dose or delay the need for radiotherapy with its inherent long-term toxicity. It should be recognised that although not only do differences exist in chemosensitivity between cultures from different patients with the same kind of brain tumour, but differences also exist between histological groups of tumour which can be demonstrated by the use of *in vitro* short-term cultures and that these differences are consistent with the differences observed in their response clinically to chemotherapy. This is the first demonstration of systematic differences in chemosensitivity between groups of brain tumours *in vitro*. The group sizes are relatively small, a reflection of the rarity of these tumours and further work is needed to confirm this differential chemosensitivity in larger groups of tumours and using a panel of drugs which includes cyclophosphamide derivatives, etoposide and platinum drugs which are now being applied more widely to childhood brain tumours. The ideal setting for this expanded study would be as part of a large-scale multicentre clinical trial in which the relationship between *in vitro* sensitivity and clinical outcome can be rigorously assessed.

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