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In vitro chemosensitivity of brain tumors to cisplatin and its analogues, iproplatin and carboplatin*

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Summary. The human tumor stem-cell assay was used to investigate the in vitro chemosensitivity of 27 evaluable samples to cisplatin and its analogues, iproplatin and carboplatin, as well as to BCNU, teniposide, vindesine, and dibromodulcitol. All agents exhibited some antitumor activity with the exception of dibromodulcitol (zero response out of 19 evaluable samples). Vindesine, BCNU, and carboplatin were the three most active compounds, with response rates of 29%, 23%, and 22%, respectively. There was a lack of complete cross-resistance between carboplatin and cisplatin as well as between carboplatin and BCNU. Our data suggest that clinical studies with carboplatin and combinations of vindesine plus cisplatin and its analogues may be worthwhile.

Introduction

Tumors of the central nervous system are relatively unusual in adults, although they account for approximately 20% of all pediatric neoplasms [15]. The basic therapeutic approach for these tumors is surgery followed by radiation [15]. The chemotherapy of brain tumors remains unsatisfactory [9]. The most commonly used agents, nitrosoureas, consistently induce response rates in the range of 20%. There is therefore a clear need for new active anticancer agents for brain tumors.

The human tumor stem-cell assay developed by Hamburger and Salmon [11] appears to have great potential for in vitro drug studies, despite a number of limitations [21]. Prospective trials support its relevance as a chemosensitivity test [2, 25]. The assay could be helpful in the planning of clinical phase II trials and in the screening of new drugs [20, 22]. The stem-cell assay has been used for human brain tumors by Rosenblum et al. [18]. In a small series of ten patients, a good correlation was observed between in vitro and in vivo response.

In our laboratory the human tumor stem-cell assay has been used to investigate the in vitro antitumor activity of two cisplatin analogues, iproplatin and carboplatin. The selection of these two agents was based on the following considerations. Cisplatin is active against brain tumors [14]; iproplatin and carboplatin are less-nephrotoxic cisplatin analogues with demonstrated antitumor activity, especially in lung and ovarian cancer [4, 23, 26]. The activity of iproplatin and carboplatin was evaluated against fresh brain tumor samples and compared with the activity of agents with known activity against these tumors, including BCNU [9], vindesine [1], and teniposide [17]. Finally, we also investigated dibromodulcitol, an hexitol derivative that is still under clinical investigation [6].

Materials and methods

Reagents. Cisplatin, iproplatin, carboplatin, and teniposide were kindly supplied by Bristol Laboratories (Syracuse, New York). Vindesine was purchased from Eli Lilly Benelux (Brussels, Belgium) and BCNU from Sintesa (Brussels, Belgium). Dibromodulcitol was obtained from Chinoin (Budapest, Hungary). Abrin was obtained from Sigma Chemical Co. (St Louis, Mo).

Preparation of cells. Solid tumors were first washed in Hanks' balanced salt solution. Then, the samples were mechanically disrupted using scissors. Cells were passed through raspers to disrupt small aggregates. Cell counting was performed in a hemocytometer and cell viability was measured by the trypan blue dye exclusion method.

In vitro exposure to anticancer agents. Stock solutions of the various anticancer agents were stored at −20° C, and sterile water was used for subsequent dilutions. Nucleated viable cells at a concentration of 3×10^6/ml were exposed to the following drug concentrations: cisplatin, 0.1–1.0 μg/ml; carboplatin, 1.0–10.0 μg/ml; iproplatin, 1.0–10.0 μg/ml; vindesine, 0.1–1.0 μg/ml; BCNU, 0.1–1.0 μg/ml; teniposide, 0.1–10.0 μg/ml; and dibromodulcitol, 0.1–2.0 μg/ml. These concentrations were based on the peak plasma concentrations observed in man [5, 12, 13, 16]. Cells were incubated with or without drug for 1 h at 37° C in Hanks' balanced salt solution. The cells were then washed twice before culture. All experiments were conducted in triplicate.

Assay for tumor colony-forming units. Cultures were performed in 35 × 10 mm petri dishes. The double agar layer was prepared as previously described [19]. Tumor-colony

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Table 1. In vitro efficacy of cisplatin, iproplatin, carboplatin, dibromodulcitol, vindesine, teniposide, and BCNU against brain tumor samples[a]

Agent	Concentration μg/ml	No. of sensitive/evaluable samples (effective concentration)	Response rate (95% confidence interval)
Cisplatin	0.1 – 1.0	2/24 (1)	8.3 (0.0 – 18.4)
Carboplatin	1.0 – 10.0	4/18 (1, 1, 10, 10)	22.2 (3.0 – 41.4)
Iproplatin	1.0 – 10.0	1/18 (1)	5.6 (0.0 – 16.1)
Dibromodulcitol	0.1 – 2.0	0/19	0.0 (–)
Vindesine	0.1 – 1.0	6/21 (1)	28.6 (9.2 – 47.8)
Teniposide	0.1 – 10.0	1/15 (1)	6.7 (0.0 – 19.3)
BCNU	0.1 – 1.0	3/13 (0.1, 1, 1)	23.1 (0.2 – 46.0)

[a] Brain tumor samples were exposed to the various agents at the indicated concentrations for 1 h at 37° C. The samples were then cultured as described in *Methods*

formation was monitored using an inverted microscope. Immediately after plating, all dishes were checked for the presence of cell aggregates. Thereafter, plates were incubated at 37° C in a 5% CO_2 humidified atmosphere and examined twice or three times per week until final counting. Small clusters of 3–20 cells appeared within 10 days and colonies consisting of ≥40 cells could be observed within 7–28 days. Final scoring of the number of colonies was generally possible during the 3rd or 4th week.

Cell kill was measured by the percentage of the mean number of colonies after drug exposure relative to the mean number of control colonies. A minimum mean of 20 colonies was required in the triplicate control plates. In vitro efficacy was defined as a ≥50% decrease in tumor colony-forming units.

Results

Forty-six tumor samples were freshly obtained. Two samples could not be adequately dissociated; one sample was lost for technical reasons. Of the 43 cultured samples, the cell yield was insufficient for drug testing in 3, 9 did not grow at all, and 4 yielded fewer than 20 colonies in the control plates, leaving 27 evaluable samples (59%). The median number of colonies was 52 (range: 0–360) and the median plating efficiency, 10.4×10^{-5} (range: 0–72).

The sensitivity to the various agents is listed in Table 1. The six responses to vindesine were observed at the concentration of 1 μg/ml. Similarly, the two responses to cisplatin were observed at the concentration of 1 μg/ml. A single sample responded to iproplatin and teniposide at 1 μg/ml. For BCNU and carboplatin, responses were observed at both concentrations investigated. For BCNU, 2 of 12 samples responded at 1 μg/ml and the single sample tested at 0.1 μg/ml responded. For carboplatin, 3 of 13 samples were sensitive at 10 μg/ml and 1 of 17, at 1 μg/ml.

Sufficient data were available to test the cross-sensitivity of 18 brain-tumor samples to cisplatin, carboplatin, and iproplatin (Table 2). The single sample sensitive to cisplatin was also sensitive to carboplatin but resistant to iproplatin. Of the 17 samples resistant to cisplatin, 3 were sensitive to carboplatin and 1 was sensitive to iproplatin. A smaller number of samples was available to evaluate the cross-resistance between BCNU and cisplatin and its analogues. Of the 13 evaluable samples, 3 were sensitive to BCNU. These 3 samples were resistant to cisplatin although 1 of the 10 samples resistant to BCNU was sensitive to cisplatin. Of the 3 samples resistant to BCNU, 2 were resistant and 1, sensitive to carboplatin. Of the 10 samples resistant to BCNU, 7 were resistant and 3, sensitive to carboplatin. Finally, all these 13 samples were resistant to iproplatin.

Table 2. Cross sensitivity of 18 brain tumors to BCNU, cisplatin (DDP), carboplatin (JM8), and iproplatin (JM9)[a]

Sensitivity to		Sensitivity to					
		BCNU		JM8		JM9	
		Yes	No	Yes	No	Yes	No
DDP	Yes	0	1	1	0	0	1
	no	3	9	3	14	1	16
JM9	Yes	0	0	0	1		
	no	3	10	4	13		
JM8	Yes	1	3				
	no	2	7				

[a] Brain tumor samples were exposed for 1 h at 37° C to the various agents. The samples were then cultured as described in *Methods*

Discussion

Our work indicates that several agents are active in vitro against brain tumor samples. The main purpose of our study was to assess the activity of cisplatin and its two analogues, iproplatin and carboplatin. Our data indicate that the three compounds have antitumor activity in vitro. Carboplatin appears to be the most active, but our sample numbers are still too small to allow a statistical comparison. BCNU was used as a reference compound in this study, since it is still the most widely used agent in the chemotherapy of brain tumors [9]. The in vitro activity of BCNU (23%) corresponds very well to the clinical activity of this agent. Vindesine and teniposide also exhibited some antitumor activity, while dibromodulcitol was inactive. The disappointing results with dibromodulcitol do not support its use as an antitumor agent for brain tumors despite its very good penetration of the central nervous system [7]. Our data also suggest a lack of complete cross-resistance between several of the investigated agents. The most striking data were obtained with carboplatin. This substance was active against 3 of 17 cisplatin-resistant samples as well as against 3 of 10 BCNU-resistant samples.

Our study could be criticized on the grounds that negative controls were not included i.e., no clinically inactive

drugs were selected. However, in the case of brain tumors, clinical inactivity may be largely related to the inability of several agents to cross the blood-brain barrier, although some of these agents may have intrinsic antitumor activity.

Our study provides a rationale for clinical studies in brain tumors with the analogues of cisplatin, especially carboplatin. The known activity of cisplatin is hampered by the toxicity of this agent [14]. In addition to the usual side-effects of cisplatin, neurological complications were more frequent in patients with brain tumors. They included seizures related to hyponatremia and a transient deterioration of the neurological status. Some of these adverse effects may be related to acute fluid retention and serum electrolyte abnormalities, secondary to the hydration programs commonly used during cisplatin chemotherapy. Iproplatin and carboplatin are less nephrotoxic compounds than cisplatin and can be administered without hydration [4, 23, 26].

The in vitro activity of vindesine and cisplatin and its analogues suggest that it might be useful to investigate the clinical antitumor activity of combinations of vindesine plus cisplatin or its analogues. In addition, the combination of cisplatin with vindesine has been reported to be at least additive in human lung cancer [10]. A classic consideration for the chemotherapy of brain tumors is the inability of several anticancer agents to cross the blood-brain barrier [8]. However, the penetration of cytotoxic agents into brain tumors may be higher than its penetration into the cerebrospinal fluid and normal brain tissue. For example, for cisplatin, the ratio of cerebrospinal fluid over plasma concentration is very low, but potentially cytotoxic concentrations of cisplatin can be detected in tumor samples obtained at autopsy or surgery [24]. Finally, animal data indicate that cisplatin, carboplatin, and iproplatin penetrate murine brain to a similar extent [3].

In conclusion, the human tumor stem-cell assay appears to be a useful tool in detecting new agents with antitumor activity in brain tumors; clinical studies should be performed to validate this hypothesis.

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