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Combination chemotherapy for choroidal melanoma: *ex vivo* sensitivity to treosulfan with gemcitabine or Cytosine arabinoside

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Summary Treatment of choroidal melanoma by chemotherapy is usually unsuccessful, with response rates of less than 1% reported for dacarbazine (DTIC)-containing regimens which show 20% or more response rates in skin melanoma. Recently, we reported the activity of several cytotoxic agents against primary choroidal melanoma in an ATP-based tumour chemosensitivity assay (ATP-TCA). In this study, we have used the same method to examine the sensitivity of choroidal melanoma to combinations suggested by our earlier study. Tumour material from 36 enucleated eyes was tested against a battery of single agents and combinations which showed some activity in the previous study. The combination of treosulfan with gemcitabine or cytosine arabinoside showed consistent activity in 70% and 86% of cases, respectively. Paclitaxel was also active, particularly in combination with treosulfan (47%) or mitoxantrone (33%). Addition of paclitaxel to the combination of treosulfan + cytosine analogue added little increased sensitivity. For treosulfan + cytosine arabinoside, further sequence and timing experiments showed that simultaneous administration gave the greatest suppression, with minor loss of inhibition if the cytosine analogue was given 24 h after the treosulfan. Administration of cytosine analogue 24 h before treosulfan produced considerably less inhibition at any concentration. While we have so far been unable to study metastatic tumour from choroidal melanoma patients, the combination of treosulfan with gemcitabine or cytosine arabinoside shows activity *ex vivo* against primary tumour tissue. Clinical trials are in progress.

Keywords: chemosensitivity; melanoma; eye; choroid; ATP; DNA repair, apoptosis; anti-neoplastic agents; combination

Choroidal melanoma is a chemoresistant tumour which is fatal in about 50% cases at 10 years and has a median survival of 5 to 7 months following the development of metastases (Ravio, 1977; Albert et al, 1992; Bedikian et al, 1995). Although a high-risk group can be defined by stage (tumour size) and microvascularity (Foss et al, 1996), no adjuvant therapy is available for routine use. Primary therapy is limited to enucleation or local irradiation (Albert et al, 1992).

Treatment of metastatic choroidal melanoma with chemotherapy using regimens applied to skin melanoma has been largely unsuccessful and has been based on the premise that because the two tumours are derived from the same cell type, they may respond similarly, despite the many genetic and phenotypic differences between them (Albert et al, 1996; Chana et al, 1998). While occasional responses have been reported, there are few trial-based data from which response rates can be obtained (Albert et al, 1992). One large series reported a response rate less than 1% for systemic therapy, although chemoembolization of the liver using cisplatin-based regimens was more effective, producing responses in 36% of patients (Cantore et al, 1994; Bedikian et al, 1995). The results of these studies suggest that at least some of these tumours are partially sensitive to platinum-based therapy, although in systemic combination with other drugs, cisplatin shows little effect (Proebstle et al, 1996).

In a recent study of the chemosensitivity of uveal melanoma ex vivo (Myatt et al, 1997), we used an ATP-based luminescence assay (Hunter et al, 1993; Andreotti et al, 1995; Cree et al, 1996; Kurbacher et al, 1998) to determine the sensitivity of primary uveal melanoma to a variety of chemotherapeutic agents. We observed variable sensitivity to treosulfan, cytosine arabinoside, paclitaxel and doxorubicin and showed enhancement of the response to treosulfan by cytosine arabinoside (Myatt et al, 1997). Recently, a new derivative of cytosine arabinoside, gemcitabine, has been shown to have greater effects on solid tumours (Plunkett et al, 1995). Previous experience with gemcitabine in modulating Cisplatin activity in ovarian cancer both clinically and in vitro was encouraging (van Moorsel et al, 1997; Kurbacher et al, 1998). As a corollary, we therefore decided to explore the use of the combination of treosulfan + gemcitabine further ex vivo by chemosensitivity testing of primary uveal melanoma tumours.

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MATERIALS AND METHODS

Melanomas

Material from 37 consecutive large primary intra-ocular melanomas (Table 1) was obtained under sterile conditions from fresh enucleation specimens removed consecutively at Moorfields

Table 1 Drug concentrations used in the assay and their clinically relevant doses. For combinations, each drug was added at the TDCs to the same wells. The dose correlation represents the standard dose from which pharmacokinetic data were used to estimate the test drug concentration and is given for information only

Drug name	Test Drug Concentration (TDC)		Drug dose Correlation
	μ ml^{-1}	μM	
Cytosine arabinoside	2.4	9.87	I.V. 100 mg m^{-2}
Doxorubicin	0.5	0.86	I.V. 60 mg m^{-2}
Treosulfan	3	10.5	ORAL 1 g day^{-1}
Vincristine	0.4	0.48	I.V. 1.5 mg m^{-2}
Vinblastine	0.5	0.62	I.V. 6.0 mg m^{-2}
Paclitaxel	6.8	7.96	I.V. 175 mg m^{-2}
Gemcitabine	12.5	47.5	I.V. 1250 mg m^{-2}
Mitoxantrone	0.3	0.58	I.V. 12 mg m^{-2}

Eye Hospital or St Bartholomew's Hospital over a 9-month period. All except two were previously untreated: the exceptions had failed local ruthenium plaque or proton beam radiotherapy. Three patients had had diagnostic choroidal biopsy preceding enucleation. Enucleated eyes were examined externally for the presence of extrascleral extension (present in four cases) or previous surgery, and by transillumination using a fibre-optic light source to locate the tumour. The eye was then oriented in a steel eye-cup and sectioned posteriorly starting at the cornea to one side of the midline continuing to the same side of the optic disc. The larger calotte was placed immediately into 4% buffered formaldehyde for histopathology, while the smaller calotte without the optic disk was examined. The tumours ranged in size (largest tumour diameter) from 7–23 mm, with a median of 12 mm. Ciliary body involvement was present in four of 37 tumours, two of which appeared to originate from the ciliary body, while the remainder were restricted to the choroid. Tumour material was scraped from the calotte and placed into 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) to which 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin had been added. The primary consideration was always to obtain a histopathological diagnosis and in cases of doubt, the bulk of the tumour was fixed for diagnostic use. Histologically, 15 tumours were classified as spindle cell, 17 as mixed and five as epithelioid tumours. The mitotic index varied from 0–12 mitoses mm^{-2}, with a median of 0.6 mitoses mm^{-2}. A further 10 tumours (nine choroidal, one ciliary body; five epithelioid, three spindle, two mixed type; mitotic index 0–2.6) were used in subsequent experiments to determine the effect of drug sequence in combination experiments. Approval for use of material not required for diagnosis was obtained from the Moorfields Eye Hospital Ethics Committee.

ATP-based tumour chemosensitivity assay (ATP-TCA)

ATP-TCA was performed as previously described (Andreotti et al, 1995; Myatt et al, 1997). This assay is based on the linear relationship between ATP content and biomass (Petty et al, 1995). Cells were dissociated from melanoma tissue by incubation overnight at 37°C with 1.5 mg ml^{-1} collagenase type H (Sigma Chemical Co Ltd., Poole, UK). The collagenase concentration was reduced to 0.75 mg ml^{-1} from tumour 36 onwards. Following dissociation, the cells were washed in an antibiotic-containing serum-free complete assay medium (CAM, DCS Innovative Diagnostik Systeme, Hamburg, Germany) by centrifugation at 400 ***g*** for 10 min and their viability checked by trypan blue exclusion. Cell viability ranged from 50% to 95% (mean 85%). Ficoll-hypaque density centrifugation (Lymphoprep, Nycomed UK Ltd, Birmingham, UK) with two further washes was used in two cases to remove cell debris. The cells were adjusted to 200 000 viable cells ml^{-1} in CAM and 100 μl added to the wells of a 96-well polypropylene microplate (Costar, High Wycombe, UK) to which doubling dilutions of four drugs in triplicate wells (in 100-μl volumes) had been added while the cells were being prepared. Test drug concentrations (TDCs) are based on pharmacokinetic data adjusted to provide good discrimination between tumours (Table 1) (Andreotti et al, 1995). Combinations used two to three drugs added simultaneously unless otherwise specified. All drugs were left in the plate for the duration of the culture period. One row was reserved for six control wells with 100 μl CAM only (MO) and six wells to which 100 μl of a maximum inhibitor of cell survival (MI, DCS Innovative Diagnostik Systeme) was added.

The plate was incubated for 6–7 days at 37°C with high humidity in 5% CO_2 and the cells observed every 2–3 days by microscopy to check for infection or overgrowth. At the end of the incubation period, ATP was extracted from the cells by addition of a detergent-based extractant (TCER, DCS Innovative Diagnostik Systeme) and 50 μl from each well was added to 3.5 ml polystyrene tubes (Sarstedt, Numbrecht, Germany) or white microtitre plates (Dynatech Ltd, Billinghurst, UK), for estimation of ATP levels by luminescence assay. The tubes were loaded into a Berthold LB953 luminometer (EG&G Berthold, Wildbad, Germany) set to inject 55 μl of luciferin–luciferase reagent (DCS Innovative Diagnostik Systeme). Light output expressed as relative light units (RLU) was used to determine the mean % inhibition of cell growth/survival in triplicate wells at each drug concentration according to the following equation: $1 - ((\text{Test} - \text{MI})/(\text{MO} - \text{MI})) \times 100$.

For subsequent sequence experiments, plates were made up with the first drug in 100-μl volumes down the plate as before and cells added in 50 μl rather than 100 μl to allow later addition of 50 μl of the second drug (final concentration = 50% TDC) at varying intervals. Cells were plated at 20 000 per well as before. Cultures were stopped, and their ATP content and the degree of inhibition were measured as usual. Six experiments were performed with cytosine arabinoside + treosulfan, and four with gemcitabine + treosulfan using primary uveal melanoma cells.

Data analysis

The results of each assay were analysed individually in an Excel 5.0 spreadsheet (Microsoft) allowing graphical representation of the response (Figure 1) and collected in a database (Access 2.0, Microsoft). Wells responsible for high variation in MO or test results were examined and excluded as outliers or known errors (e.g. pipetting, excessive cell clumping). For comparison of responses between patients (Table 2), a simple logarithmic index was derived by summing the percentage inhibition at each level of TDC tested as $\text{Index} = 700 - \text{Sum}[\text{Inhibition}_{3,13 \ldots 200}]$ (Hunter et al, 1993). An arbitrary level of 50% inhibition (Index < 350), IC_{50} and IC_{90} were used to assess relative *ex vivo* sensitivity or resistance (Table 3). Combination effects were assessed for independent and combination effects using the method described by Poch et al (1990).

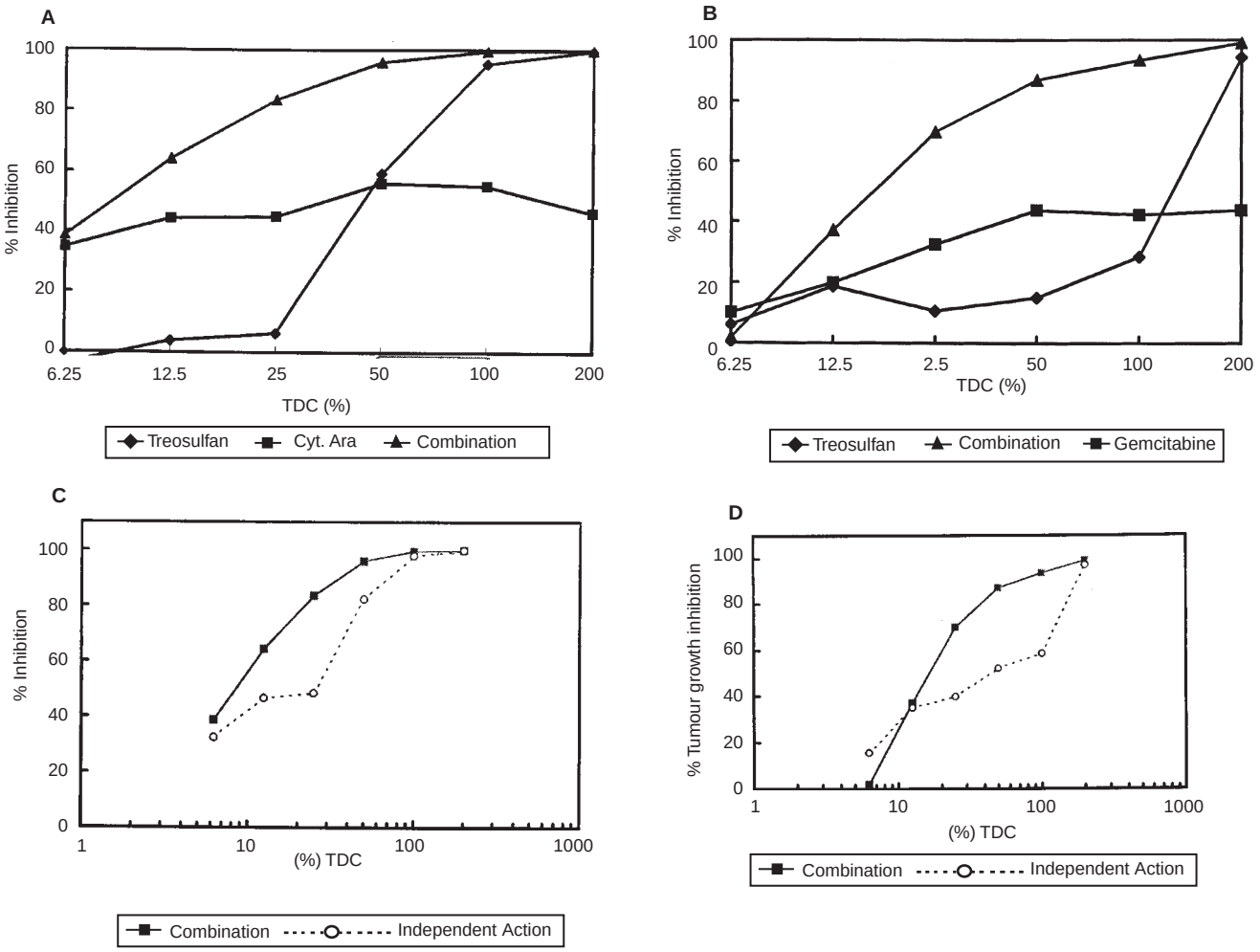

Figure 1 Example ATP-TCA results for the combination of treosulfan with (**A**) cytosine arabinoside (case 15) and (**B**) gemcitabine (case 30) with combination effect graphs (Poch et al, 1990) for (**C**) treosulfan + cytosine arabinoside and (**D**) treosulfan + gemcitabine. In both sets of results, there is some sensitivity to both cytosine analogues at all concentrations tested, but this never reaches 100% inhibition. Treosulfan sensitivity is poor at most concentrations, but there is greater than 90% inhibition at high concentrations. However, in both cases, the combination is much more effective. TDC = test drug concentration

RESULTS

Evaluable results were obtained from 31 of the 37 melanomas tested, giving an evaluability rate of 84%. Six tumours were non-evaluable: three had low MO values indicating death of most cells (without drug present) and three yielded too few cells after dissociation for assay (in one of these, the tumour was necrotic). No infections were encountered before or during culture.

The single agent results (TCA Index) are detailed in Table 3A, and the combination results in Table 3B to show the degree of heterogeneity in responsiveness to individual drugs/combinations between tumours. While most responded to treosulfan + cytosine analogue, these cases showed considerable variability in their response to other drugs. Table 2 gives a summary of the sensitivities using an arbitrary index of 350 as the cut-off below which the agent/combination was said to be sensitive, together with the number of cases in which the IC_{50} and IC_{90} fell within the range of concentrations tested. In this study, the treosulfan sensitivity was lower than previously observed (Myatt et al, 1997), although there was a dose response in most cases evidenced by the IC_{50} and IC_{90} values. Figure 1A shows a typical result from one tumour for the single agents and combination of treosulfan with cytosine arabinoside (Ara-C). This indicates modulation of the alkylating agent response by Ara-C, as observed previously (Myatt et al, 1997). Similar results were obtained with gemcitabine (Figure 1B), although gemcitabine alone exhibited a slightly more pronounced dose–response curve and showed less activity at low concentrations. The combination–response curves (Poch et al, 1990, 1995) suggest a greater effect of combining the two drugs than their independent actions would predict at most concentrations, particularly in the mid-range (Figure 1C and 1D).

While most tumours showed a response to treosulfan which could be modulated by cytosine analogues, there was considerable variation between individuals in the response to other single agents (Table 3). There was no relationship between sensitivity index for any of the drugs listed and mitotic rate, tumour size or cell type. Many tumours showed a response to cytosine arabinoside (43%) or gemcitabine (30%) as single agent. However, these drugs never induce 100% cell kill when present alone. Sensitivity to anthracyclines was observed in 7% of cases tested with doxorubicin and 11% tested with mitoxantrone. There was some indication of a lack of cross-resistance between these two

Table 2 Summary of results using an arbitrary threshold for sensitivity of Index < 350, and the number of cases in which the IC_{50} and IC_{90} fell within the range of concentrations tested (3.13–200% TDC). Treosulfan is the most active alkylating agent tested and the combinations with cytosine arabinoside or gemcitabine are effective in most tumours tested. The median and range (in brackets) for each parameter in terms of TDC% are shown below, where these fall within the concentration range tested. na = not achieved.

Drug	Sensitivity index	IC_{50}	IC_{90}
Cytosine arabinoside[a]	12/28 (43%)	19/29 (66%)	1/29(3%)
	387 (63–875)	5 (4–60)	na
Doxorubicin	2/27 (7%)	10/23 (43%)	0/23 (0%)
	494 (307–1309)	92 (9–159)	na
Treosulfan	2/31 (6%)	18/31 (58%)	6/31 (19%)
	491 (191–1003)	105 (3–163)	21 (14–53)
Vincristine/vinblastine	0/14 (0%)	0/14 (0%)	0/14 (0%)
	682 (482–1011)	na	na
Paclitaxel	4/29 (14%)	13/33 (39%)	1/33 (3%)
	525 (189–816)	109 (3–186)	199
Gemcitabine	6/20 (30%)	8/20 (40%)	0/20 (0%)
	480 (237–918)	85 (4–143)	na
Mitoxantrone	1/9 (11%)	5/9 (56%)	0/9 (0%)
	384 (258–514)	112 (98–157)	na
Mitoxantrone + Paclitaxel	3/9 (33%)	9/9 (100%)	3/9 (33%)
	373 (258–485)	134 (101–170)	32 (22–40)
Doxorubicin + Paclitaxel	8/26 (31%)	20/26 (77%)	9/26 (34%)
	400 (233–657)	125 (3–180)	30 (22–39)
Treosulfan + Ara C	25/29 (86%)	27/29 (93%)	20/29 (69%)
	214 (28–611)	3 (3–175)	41 (6–121)
Treosulfan + Gemcitabine	16/23 (70%)	22/23 (96%)	18/23 (78%)
	306 (61–698)	104 (3–161)	28 (6–72)
Treosulfan + Doxorubicin	1/8 (13%)	7/8 (88%)	3/8 (38%)
	433 (178–625)	107 (3–158)	65 (28–73)
Treosulfan + Paclitaxel	8/17 (47%)	16/17 (94%)	13/17 (76%)
	353 (131–567)	126 (3–175)	32 (20–88)
Treosulfan + Paclitaxel + Ara C	11/12 (92%)	11/11 (100%)	11/11 (100%)
	148 (36–351)	3 (3–134)	43 (6–73)
Treosulfan + Paclitaxel + Gemcitabine	9/11 (82%)	11/11 (100%)	10/11 (91%)
	286 (189–456)	105 (3–194)	33 (24–43)

[a]100% melanoma cell kill not achieved.

anthracyclines with generally greater sensitivity to mitoxantrone. Fourteen per cent of cases showed sensitivity to paclitaxel, but there were no responses to vinca alkaloids (vincristine or vinblastine). The combination of paclitaxel with doxorubicin showed activity in 31%, while mitoxantrone + paclitaxel was effective in 33%.

Treosulfan + cytosine arabinoside was the best of the double-agent combinations, with 86% of patients showing sensitivity. The combination of treosulfan with gemcitabine showed similar efficacy in 70% cases. While the highest sensitivity was seen for the combination of treosulfan + paclitaxel + cytosine arabinoside (92%), in general paclitaxel added little to the sensitivity of the combination (Figure 2A). However, in occasional patients with limited treosulfan sensitivity and some paclitaxel sensitivity, paclitaxel addition does improve the response (Figure 2B).

Schedule experiments combining treosulfan at different times with cytosine arabinoside or gemcitabine are shown in Figure 3. These experiments were conducted with 50% TDC concentrations of cytosine arabinoside or gemcitabine and show simultaneous addition to be most effective. Prior addition of gemcitabine or cytosine arabinoside before treosulfan abrogated the effect of the alkylating agent and in this sequence the combination failed to produce 100% inhibition.

DISCUSSION

In the UK, rare tumours (i.e. those outside the top 10 for incidence) account for 25% of deaths from cancer (Thames Cancer Registry, 1995). Choroidal melanoma is rare with an age-standardized incidence of 0.4 to 1.2 cases per 100 000 within Europe (Foss and Dolin, 1996). Consequently, clinical trials are difficult and rarely performed as funding bodies, whether industrial or charitable, prefer to concentrate resources on more common tumours. Yet these patients need treatment. Chemosensitivity testing has a poor reputation following a long history of technical problems. However modern methods offer a way of testing new drugs and regimens in rare tumours which could not be contemplated in the clinical setting. ATP-based methods have theoretical advantages of sensitivity and reproducibility over other methods (Petty et al, 1995; Cree and Kurbacher, 1997). The ATP-TCA overcomes most of the difficulties which have beset chemosensitivity testing and shows considerable promise as a way of individualizing chemotherapy (Cree and Kurbacher, 1997). Correlation of sensitivity in the ATP-TCA with clinical response is comparable with bacteriological or oestrogen receptor testing at 75–80% (Andreotti et al, 1995; Cree et al, 1996; Cree and Kurbacher, 1997). For choroidal melanoma, our evaluability rate has improved from 71%

Table 3 Results of testing for each tumour expressed as a simple summary index of inhibition across the range of concentrations tested for (**A**) single agents and (**B**) Paclitaxel and combinations. Low values indicate considerable inhibition, while higher values indicate resistance. Values greater than 700 indicate growth greater than control wells, which is likely to be artefactual and simply reflects resistance. nd = not done.

(A)

Number	Cytosine arabinoside	Doxorubicin	Treosulfan Vinblastine[a]	Vincristine	Paclitaxel	Gemcitabine	Mitoxantrone
1	685	465	539	671	465	nd	nd
3	641	429	510	682	220	nd	nd
4	752	606	720	604	393	nd	nd
5	820	307	739	725	407	nd	nd
7	248	449	380	nd	410	nd	nd
8	653	539	566	482	525	nd	nd
9	274	633	402	nd	476	nd	nd
11	181	320	600	592	189	273	nd
12	63	453	491	nd	464	237	nd
13	196	501	507	759	500	284	nd
14	180	337	462	nd	478	nd	nd
15	367	541	420	nd	556	nd	nd
16	423	615	364	633	580	529	nd
17	670	464	623	613	581	605	nd
18	183	382	191	nd	582	nd	nd
19	379	513	449	767	329	587	nd
20	453	638	521	891	816	918	nd
21	480	nd	397	nd	327	nd	nd
23	396	409	456	726	569	589	nd
24	627	781	1003	1011	nd	678	nd
26	nd	nd	389	nd	607	460	nd
27	498	654	472	nd	653	501	384
28	nd	nd	345	nd	530	429	339
29	320	494	446	nd	611	638	442
30	601	475	520	nd	594	515	361
31	173	529	400	nd	442	328	373
33	331	611	522	nd	541	456	258
34	nd	nd	633	nd	nd	330	nd
35	875	1309	520	nd	677	576	485
36	261	476	573	nd	640	414	406
37	311	483	429	nd	439	324	514

[a]Vinblastine used from 97M011 onwards.

(B)

Number	Mitoxantrone + Paclitaxel	Doxorubicin + Pacltaxel	Treosulfan + Ara C	Treosulfan + Gemcitabine	Treosulfan + Doxorubicin	Treosulfan + Paclitaxel	Treosulfan + Paclitaxel + Ara C	Treosulfan + Paclitaxel + Gemcitabine
1	nd	nd	nd	nd	nd	nd	nd	nd
3	nd	334	250	nd	nd	nd	nd	nd
4	nd	357	464	nd	nd	nd	nd	nd
5	nd	284	611	nd	nd	nd	nd	nd
7	nd	462	206	nd	nd	nd	nd	nd
8	nd	403	582	nd	nd	nd	nd	nd
9	nd	347	148	nd	nd	nd	nd	nd
11	nd	233	73	210	178	131	119	nd
12	nd	399	28	61	461	375	36	nd
13	nd	401	99	100	625	302	68	nd
14	nd	293	90	130	nd	nd	54	nd
15	nd	513	191	228	nd	nd	173	nd
16	nd	483	242	387	543	334	242	nd
17	nd	424	513	374	406	368	302	nd
18	nd	441	98	86	nd	nd	123	nd
19	nd	283	214	315	406	202	257	nd
20	nd	657	312	378	496	567	351	nd
21	nd	nd	203	nd	nd	nd	118	nd
23	nd	367	257	431	391	291	180	nd
24	nd	nd	303	698	nd	nd	nd	nd
26	nd	nd	260	367	nd	nd	nd	402
27	384	640	275	343	nd	555	nd	319
28	339	487	125	237	nd	353	nd	249
29	442	459	140	218	nd	392	nd	198
30	361	400	276	312	nd	461	nd	286
31	373	311	113	273	nd	284	nd	189
33	258	304	160	339	nd	336	nd	336
34	nd	nd	nd	245	nd	nd	nd	228
35	485	565	281	389	nd	463	nd	456
36	337	514	251	306	nd	470	nd	311
37	426	263	129	254	nd	222	nd	221

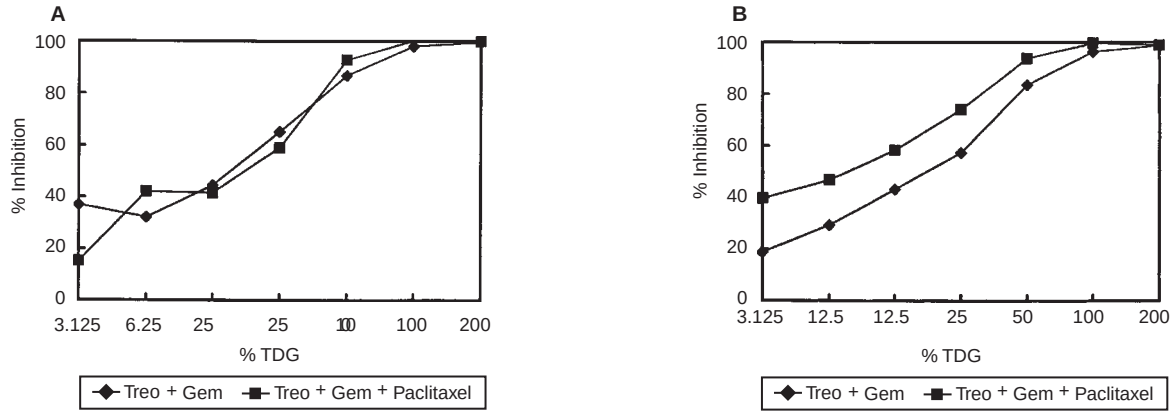

Figure 2 Examples showing addition of paclitaxel to the combination of treosulfan + gemcitabine. In case 28 (**A**) there is no effect, while in case 31 (**B**) a small effect is observed. TDC = test drug concentration

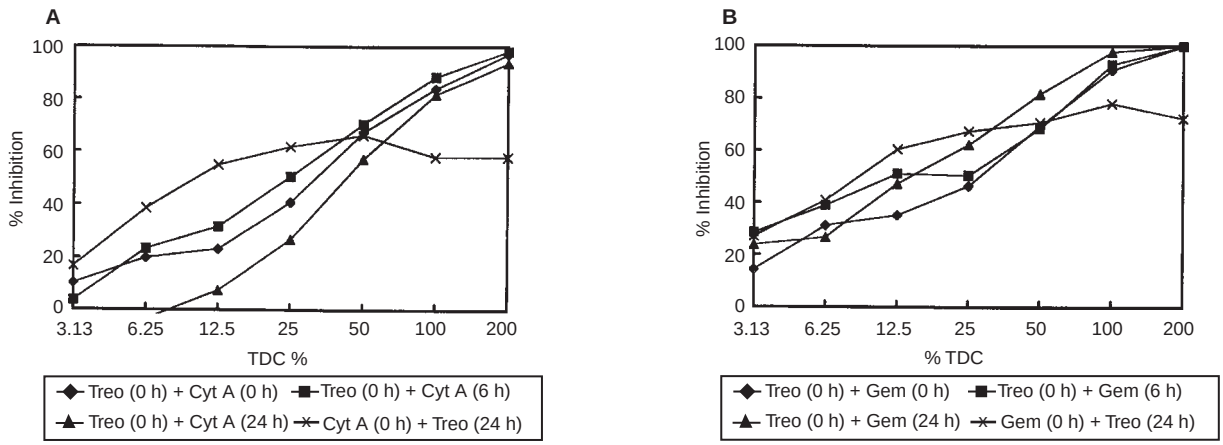

Figure 3 Sequential studies with (**A**) cytosine arabinoside and (**B**) gemcitabine in combination with treosulfan. When either cytosine analogue is given before the treosulfan, the combination fails to acheive 100% inhibition. In both cases, best inhibition is achieved by concomitant administration of the two drugs. TDC = test drug concentration

in the first 28 cases tested (Myatt et al, 1997) to 86% in this series (the current series includes four of the first series tumours in which combinations were tested). The improvement probably reflects the gentler dissociation and cell handling used in comparison with our initial practice (Myatt et al, 1997).

Our previous (Myatt et al, 1997) and current results confirm the chemoresistance of choioidal melanoma, but also suggest that there are options for improvement of response rates. The current study shows few differences from our previous study. Although the sensitivity of the melanomas to treosulfan was reduced (Table 2), examination of the individual data shows that most tumours (Table 3) did exhibit some sensitivity to this agent, which remains the best of the alkylating agents we have tried in the assay. We have previously shown that these tumours are relatively unresponsive to temozolomide (Myatt et al, 1997), a drug closely related to dacarbazine (DTIC) which forms the mainstay of treatment for cutaneous melanoma, but has poor results against uveal melanoma (Bedikian et al, 1995). Results for other agents were similar to those obtained previously, with confirmation of some sensitivity of uveal melanoma to paclitaxel, cytosine arabinoside and anthracyclines. As before, vinca alkaloids showed little effect.

There is heterogeneity of chemosensitivity (Table 2), as we have previously observed in many tumour types (Hunter et al, 1993; Andreotti et al, 1995), but on the basis of these results many previously untreated uveal melanomas are likely to respond to the combination of treosulfan with cytosine analogues. However, it should be noted that we have tested primary tumour material, not metastases, and it is possible that these may show differences in chemosensitivity. For clinical use, we have chosen to pursue the combination of gemcitabine + treosulfan, as the pharmacokinetics and activity of gemcitabine in solid tumours are superior to cytosine arabinoside. Preliminary clinical use of this combination in 18 heavily pre-treated patients with recurrent ovarian or breast carcinoma based on sensitivity in ATP-TCA is encouraging (data not shown) with a good safety profile and we believe that it may have wider applicability. A phase I/II trial of treosulfan + gemcitabine in metastatic uveal melanoma is now in progress, and we have commenced a phase II trial of assay-directed chemotherapy in melanoma patients with accessible disease, based on this data and studies of skin melanoma (Neale et al, unpublished data).

The differences we have observed between gemcitabine and cytosine arabinoside are interesting. Although these agents show

close cross-sensitivity, and both are cytosine analogues, there are major differences in their pharmacology (Peters et al, 1996). Several biochemical explanations are possible, but there is little doubt that gemcitabine is a useful drug in combination with alkylating agents in particular (Peters et al, 1996; Iwasaki et al, 1997; Bruckner HW, personal communication). The explanation for the apparent modulation of treosulfan sensitivity observed with both drugs may be due to inhibition of DNA repair of alkylating agent-induced cross-links, to direct incorporation of the analogues into DNA, to changes in dNTP pools (Peters et al, 1996; Iwasaki et al, 1997), or to a combination of several of these mechanisms. Further studies using assays of DNA repair and measurement of dNTP pools are required, but our results show clear evidence of synergy between the alkylating agent and cytosine analogue in primary uveal melanoma for both drugs given simultaneously.

Analysis of the single agent responses confirms our previous data showing a lack of activity of vinca alkaloids in this tumour, but some activity for paclitaxel. We had hoped that addition of paclitaxel to the combination of treosulfan with cytosine analogues might further improve responses, but in general the data do not support addition of this agent which would also add considerable toxicity. Occasional patients respond better to paclitaxel + anthracyclines than treosulfan + cytosine analogues. This was observed in three of the early cases studied (Table 3B) and it is clear from our data that there may be a role for paclitaxel and anthracyclines in patients refractory to treosulfan + cytosine analogue. Routine chemosensitivity testing to individualize testing is probably not appropriate in these patients, though it is certainly feasible, and current phase III trials of the technology may alter this view.

In this study, the ATP-TCA has shown itself able to predict combinations suitable for use in rare solid tumours in which there is no prospect of doing the number of phase II trials which would be required using current empirical methods. As the number of agents available continues to grow, the need for some form of preclinical planning of phase II trials becomes more apparent. The method used here allows large numbers of mechanistically logical permutations to be tested with material from small numbers of patients with potential benefits in terms of development time and expense.

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