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Growth and Chemotherapy of a Human Germ-Cell Tumour Line (GCT 27)

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Abstract—*The human germ-cell tumour cell line GCT 27 growing as subcutaneous xenograft tumours in male nude mice was used in the 4th and 5th passage to study chemotherapeutic drug responses. Recipient mice received* 5 Gy *whole body irradiation immediately before tumour transplantation. The median take rate was* 62% *(range* 39–73%*) and the median volume doubling time* 14 *days (range* 7–28 *days). For bleomycin, cisplatin and carboplatin a clear dose response for growth delay was observed. Bleomycin caused substantial weight loss at doses above* 75 mg/kg *whereas good response to cisplatin was obtained without serious toxic effects. Vinblastine and etoposide exerted no effect when given in non-toxic doses. The response to etoposide was not improved either by fractionated treatment or by combination with verapamil. However, the combination of* 20 mg/kg *etoposide and* 2 mg/kg *cisplatin, which when given alone were ineffective, led to a growth delay that was equal to that observed following the administration of higher cisplatin doses. This effect may be explained by the fact that etoposide, as an inhibitor of DNA-topoisomerase II, may interfere with the repair of DNA interstrand cross-links caused by cisplatin.*

INTRODUCTION

SEVENTY-FOUR PER CENT of patients with disseminated testicular cancer can be rendered disease-free when treated with the cisplatin, vinblastine and bleomycin (PVB) regimen [1]. However, this treatment is associated with considerable toxicity. Cisplatin causes severe emesis and is nephrotoxic, vinblastine produces myalgia, anaemia, thrombocytopenia and leukopenia and bleomycin's most serious adverse effect is lung damage. It is therefore important to introduce better tolerated drugs without loss of therapeutic effectiveness.

Since a close relationship between testicular cancer xenografts and their parent tumours has been demonstrated [2], we have studied the therapeutic response and toxicity of a number of chemotherapeutic agents in a human germ-cell tumour xenograft and attempted measures to improve their effectiveness.

MATERIALS AND METHODS

Mice

Male outbred nude mice (20–30 g), supplied from the Institute of Cancer Research breeding facilities, were maintained in positive pressure isolators before and in negative pressure isolators after tumour transplantation under standard lighting conditions with food and water freely available. Food and bedding were sterilized by irradiation (2500 Gy) and water autoclaved before use.

Tumour

The tumour used in this study was an orchidectomy sample of a 30-year-old patient with a malignant teratoma that contained undifferentiated (MTU), intermediate (MTI), yolk sac and seminomatous elements, stage 4B L1 according to the Royal Marsden Hospital Staging System [3]. Serum of this patient was positive for AFP and βHCG. The patient received four cycles of chemotherapy with carboplatin, etoposide and bleomycin (JEB). Thereafter lung metastases were removed and viable MTU was found. After two further cycles of JEB the patient has subsequently remained in remission.

From the orchidectomy sample Dr. Martin Pera of this department raised the human germ-cell tumour cell line GCT 27. Four to 6 months following the subcutaneous injection of 2×10^6 GCT 27 cells tumours appeared in the flanks of male nude mice. Tumours were passaged by implanting $2 \times 2 \times 2$ mm fragments s.c. into both flanks of male nude mice that had received 5 Gy whole body

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irradiation (^{60}Co, 0.6 Gy/min) immediately before transplantation. It has been shown that take rate can be increased and latency period shortened by further immunosuppression of nude mice by X-irradiation [4].

Chemotherapeutic studies

For chemotherapeutic studies tumours between 0.2 and 0.5 g were used, with five tumours per experimental group. All experiments were repeated at least once. Commercially available preparations of bleomycin (Lundbeck Ltd., Luton, U.K.), vinblastine (Eli Lilly, Basingstoke, U.K.), etoposide (Mead-Johnson, Langley, Slough, U.K.) and verapamil (Knoll Pharmaceuticals, U.K.) were used. Bleomycin was dissolved in 0.9% saline, and vinblastine, etoposide and verapamil were diluted with 0.9% saline. Cisplatin (Drug Development Branch, National Cancer Institute, Bethesda, MD, U.S.A.) and carboplatin (kindly provided by Dr. K. Harrap, Institute of Cancer Research, Sutton, Surrey, U.K.) were also dissolved in 0.9% saline. All drugs were freshly made up for each experiment and injected intraperitoneally (i.p.) at a concentration such that the mice received 0.01 ml/g body weight.

Tumour measurements

Tumours were measured once a week, in two dimensions at right angles to each other using vernier calipers. Tumour weight was calculated using a calibration curve derived for tumours in nude mice by the method of Steel and Adams [5]. Tumour response was measured as the median time to regrow to twice the pretreatment volume ($T2\times$) as a function of drug dose.

Toxicity

The death of mice during the experiments was recorded and the cumulative deaths at termination of the experiments was used as a measure of toxicity. Non-lethal effects on the mice were evaluated by body weight determinations at 2–3 day intervals. We then used a computer program to calculate for each mouse the average weight deficit (AWD) over the 14 day period following treatment. This was calculated as the first moment of rotation of the polygon describing body weight to 14 days. The mean AWD was then found for each group of five mice.

RESULTS

The median tumour take rate was 62% (range 39–73%). One passage was performed without whole body irradiation of the mice beforehand. Take rate in that instance was 35% which is below the range of take rates found when tumours were implanted in irradiated animals.

The median latency period, defined as the time taken for the tumour xenografts to reach treatment size (0.2 g) from implantation, was 35 days (range 25–65 days).

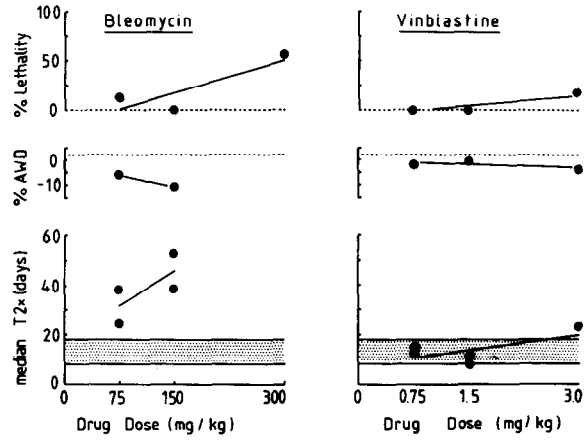

Fig. 1. The response of GCT 27 tumours to bleomycin and vinblastine. T 2× indicates the time to grow to twice the treatment volume (shaded area covers the range from lower to upper quartiles). Toxicity is indicated by percentage lethality and the average weight deficit at 14 days, AWD, expressed as a percentage of body weight at the time of treatment (broken lines show control values).

The median tumour volume doubling time ($T2\times$) was 14 days (range 7–28 days).

The results obtained when the tumour-bearing mice were treated with bleomycin or vinblastine are shown in Fig. 1. It can be seen that bleomycin had a marked effect on tumour growth, but this was accompanied by substantial toxicity in terms of weight loss. The AWD at a dose of 150 mg/kg was about 10%. This is a very severe effect in mice and indeed the maximum weight loss at this dose was 18.2± 1.6%. At the higher dose of 300 mg/kg lethality reached 57% and tumour response was not evaluable. All bleomycin-induced deaths occurred within 7 days of bleomycin administration.

Vinblastine produced no significant anti-tumour effect. However, 3 mg/kg of vinblastine resulted in 17% lethality (within 5 days) and an average weight deficit of 4%.

The results obtained from mice treated with cisplatin or carboplatin are summarized in Fig. 2. For cisplatin a marked effect on tumour growth was observed at doses of 3.5 or 5 mg/kg i.p. However a tight threshold dose for this effect was observed, no response being recorded at 2 mg/kg. Furthermore, these good therapeutic responses produced by cisplatin were obtained with no obvious signs of normal tissue toxicity. The highest dose we were able to test using growth delay as the endpoint was 5 mg/kg, half the LD_{10} dose in immunosuppressed mice [6], because in the first experiment we performed with that dose four of five tumours disappeared completely.

Carboplatin also produced a marked effect on tumour growth although some toxicity was observed with this compound; lethality for 80 mg/kg car-

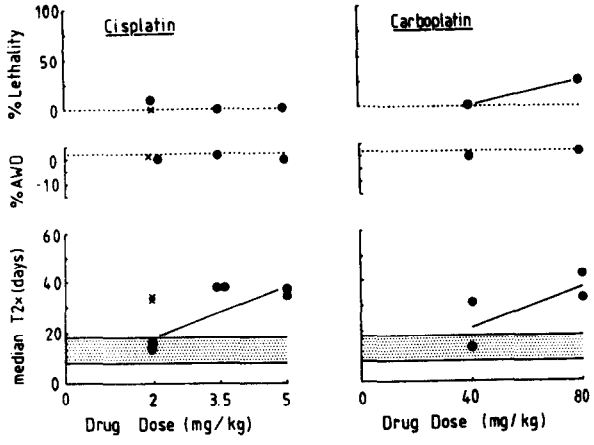

Fig. 2. The response of GCT 27 tumours to cisplatin and carboplatin. See legend to Fig. 1 for details. The solid circles show single i.p. doses of cisplatin or carboplatin and the crosses show the tumour response following the combined i.p. administration of 2 mg/kg *cisplatin and* 20 mg/kg *etoposide.*

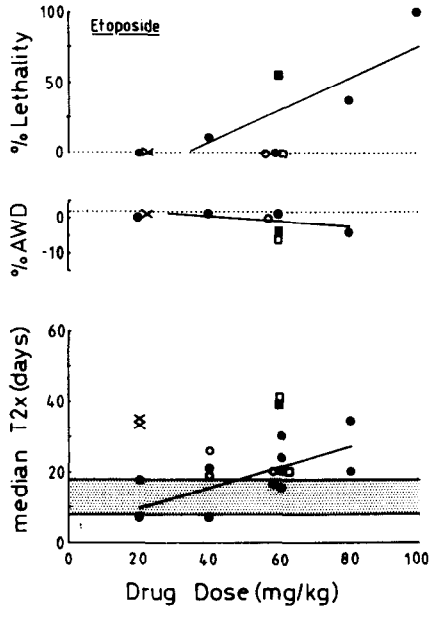

Fig. 3. The response of GCT 27 tumours to etoposide. See legend to Fig. 1 for details. The solid circles show single i.p. injections, open circles three daily i.p. injections, solid squares single combined administration of verapamil and etoposide and open squares three daily combinations of verapamil and etoposide. Verapamil dose was 25 mg/kg. *Crosses show the combined i.p. administration of* 20 mg/kg *etoposide and* 2 mg/kg *cisplatin.*

boplatin was 25% within 6 days.

Figure 3 summarizes the tumour response found when mice were treated with etoposide (VP 16). When given as a single i.p. injection at 20 or 40 mg/kg, etoposide had no effect on tumour growth. Increasing the single dose of etoposide to 60 or 80 mg/kg did lead to a small effect. However, lethality for 80 mg/kg was 38% and for 100 mg/kg 100% which is consistent with a reported LD_{10} in immunosuppresssed mice of 40 mg/kg [6]. In an attempt to improve the effectiveness of etoposide, the drug was administered as three daily injections of either 13 or 20 mg/kg. Pharmacokinetic studies in Lewis lung carcinoma bearing mice have demonstrated that at the dose of 13 mg/kg etoposide tumour levels were relatively higher than after 40 mg/kg [7]. However, splitting the etoposide dose in this way did not result in any significant benefit compared to the single i.p. injections.

The calcium antagonist verapamil, at a dose of 25 mg/kg, has been shown to potentiate the effect of etoposide in acute lymphatic leukaemia [8]. Therefore, we administered both drugs concomitantly as single i.p. injections or as three daily doses, the results of which are shown in Fig. 3. However, as can be seen, neither combination potentiated etoposide action in GCT 27 but lethality of the combined single i.p. treatment increased to 56%. Verapamil alone (data not shown) exhibited no effect on tumour growth. All deaths recorded following etoposide administration occurred between 2 and 39 days, median 11 days.

There is some evidence of synergistic activity between cisplatin and etoposide in advanced P388 leukaemia [9]. We therefore administered a dose of 20 mg/kg etoposide together with 2 mg/kg cisplatin and although both doses when given alone were ineffective the combined treatment led to equal growth delay comparable with that seen after higher cisplatin doses (Figs 2 and 3).

DISCUSSION

Human testicular cancers when grown as xenografts retain some of the distinguishing features of this disease [2]. Volume doubling times of testicular tumour xenografts from 1.6 to 12 days have been reported previously [2, 6, 10–13]. The volume doubling time of 14 days found in the present study for GCT 27 is therefore consistent with the higher of these estimates. Doubling times between 9.4 and 14.5 days also have been reported for colon, breast, lung, melanoma and ovarian tumour xenografts [6].

A 52% objective response rate was reported when metastatic germinal neoplasms of the testis were treated with vinblastine alone [14]. However, the lack of effectiveness of vinblastine alone observed in the present study in the GCT 27 tumour system is in line with previous studies using testicular xenografts [6, 12, 13]. When vinblastine was combined with bleomycin 74% of stage III testicular cancer patients responded [15]. In the treatment of the GCT 27 xenograft bleomycin also showed pronounced antitumour activity. This effectiveness of bleomycin in testicular cancer xenografts has also been observed by Steel *et al.* [6] working with a number of lines and by Osieka *et al.* [12] employing the testicular cancer xenograft line Ma. Lapis *et al.* [13] reported a small growth delay (1.5 days) for bleomycin in his TT2 line but the dose of bleomycin

he used (15 mg/kg) is difficult to compare with the doses (75 and 150 mg/kg) we used. However, the anti-tumour activity of bleomycin observed here and previously was also associated with some degree of toxicity, e.g. weight losses of −6% and −11% for 75 mg/kg and 150 mg/kg respectively. The toxicity of bleomycin is well known clinically and a recent trial comparing the effectiveness of combined etoposide and cisplatin with or without bleomycin, in small volume metastatic testicular cancer, concluded that the reduction in toxicity found in the absence of bleomycin was also accompanied by a decrease in tumour response [16].

The introduction of cisplatin in the treatment of testicular cancer made complete remission rates of 70% possible. Cisplatin was also the most effective of the drugs tested in the GCT 27 xenograft with complete disappearance of tumour with doses of as little as half the LD_{10} dose. Furthermore, the threshold for the tumour dose–response curve we obtained for cisplatin was narrow with no effect at 2 mg/kg and maximal effect at 3.5 mg/kg cisplatin. Carboplatin, the second platinum analogue tested, was also effective but with associated toxicity unlike cisplatin.

Etoposide which has been shown to induce remissions in 46% of advanced testicular tumours [17] was completely ineffective in GCT 27 at doses of 20 and 40 mg/kg but there was evidence of a response above 60 mg/kg. Osieka *et al.*[12], using a dose of 15 mg/kg, and Steel *et al.* [6], using 40 mg/kg, the LD_{10} dose for immunosuppressed mice, also reported a lack of activity of etoposide in testicular cancer xenografts. The administration of a total dose of 40 mg/kg of etoposide as three daily doses did not improve its effectiveness although pharmacokinetic studies showed that there are relatively higher tumour levels of etoposide after a dose of 13 mg/kg than following a dose of 40 mg/kg [7].

The calcium blocking agent verapamil has been demonstrated to augment etoposide accumulation, and to potentiate etoposide-induced DNA damage and cytotoxicity in L1210 cells *in vitro* [18, 19]. However, neither the combined administration of verapamil with single doses of etoposide nor the combined fractionated administration led to a reproducible enhancement of the etoposide effect. The results obtained with the combination of 60 mg/kg etoposide and 25 mg/kg verapamil are difficult to interpret, moreover, because in one experiment the majority of animals died due to toxicity. The combination of 20 mg/kg etoposide with 2 mg/kg cisplatin (both doses being ineffective when given alone) produced a growth delay equal to that seen after 3.5 and 5 mg/kg of cisplatin. Furthermore, this effect was not associated with any signs of toxicity and suggests a possible synergistic effect between the compounds.

The cytotoxicity of cisplatin has been correlated with the formation of DNA interstrand cross-links [20] whereas the mechanism of cytotoxicity by etoposide appears to involve DNA-topoisomerase II mediated DNA cleavage [21, 22]. It is probable that the final outcome of drug–cell interaction depends on the repair of drug-induced damage. Since DNA-topoisomerase II is an enzyme that controls and modifies the topological states of DNA by transiently breaking a pair of complementary strands and passing another double stranded segment [23], it is possible that the observed interaction between cisplatin and etoposide may have resulted from inhibition of repair of the cisplatin-induced damage by etoposide.

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