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Chemosensitivity of Murine Renal Carcinoma*

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Summary. A non-endocrine dependent, spontaneous carcinoma of the kidney in a Wistar-Lewis rat has been studied for sensitivity to chemotherapeutic agents. Two tumour models have been employed. Subcutaneously transplanted flank nodules were used to screen single agents for antitumour acitivity. A model of intraperitoneal metastatic disease was employed to test further agents which had demonstrated some effectiveness in the nodule model. Single agents that proved ineffective were streptozotocin, neocarzinostatin, chlorozotocin and carminomycin. 5-FU, bleomycin and hydroxyurea were also ineffective at the doses tested. Agents that were effective included cyclophosphamide, adriamycin, vinblastine, vindesin and maytansine. The most effective combination therapy appeared to be cyclophosphamide with vindesin and cisplatin.

Key words: Renal carcinoma, Chemosensitivity, Animal model.

Introduction

The lack of effective chemotherapy for patients with carcinoma of the kidney has stimulated a number of studies of murine models of this disease. [2–4, 8, 11–13]. We have previously reported trials in a spontaneously arising renal carcinoma in a male Wistar-Lewis rat [2, 3]. This report reviews our previous observations of the chemosensitivity of this neoplasm and presents observations relating to the response of this tumour to additional chemotherapeutic compounds, both singly and in combination.

Materials and Methods

All of the studies were conducted on a transplantable spontaneously arising renal carcinoma in a male Wistar-Lewis rat. Two transplantation techniques were employed to mimic clinical circumstances of a solitary tumour nodule versus diffuse metastatic disease. The details of the transplantation techniques employed to develop the two animal models have been described previously (deVere White et al. 1978). The percentage of successful tumour transplantations was constant in both techniques regardless of the tumour generation employed, but the size of the resultant tumour varied with different tumour generations. For this reason, each chemotherapeutic trial was instituted using a single tumour generation with its own group of control animals and, in any single group, only one tumour generation was used to supply the transplant for all animals used in that trial.

Tumour Nodule Model

Tumour was transplanted subcutaneously (0.06–0.08 g) into the flank of male Wistar-Lewis rats (150–200 g) under ether anaesthesia. Three weeks following implantation 90% of animals had palpable tumours. All chemotherapeutic agents were administered by intraperitoneal injection (IP), commencing one week following tumour transplantation. All animals were sacrificed at week 4. Tumour was removed and wet weight of tumour recorded. A single tumour generation was employed for each set of experiments and all tumour weights were compared to controls from the same generation, rather than historical controls. The agents tested were: a) neocarzinostatin (100 u/kg weekly X 3 and 2,000 u/kg weekly X 3); b) carminomycin (0.5 mg/kg weekly X 3 and 1 mg/kg weekly X 3); c) chlorozotocin (40 mg/kg weekly X 2); d) bleomycin (15 u/kg weekly X 3); e) vindesin (1 mg/kg weekly X 2) and f) maytansine (0.425 mg/kg week 1 and 0.175 mg/kg week 3).

Metastatic Tumour Model

Male Wistar-Lewis rats (150–200 g) underwent splenectomy under ether anaesthesia and 0.06–0.08 g tumour was placed intraperitoneally. A single animal was used for transplant donation so that each experiment was conducted with control animals implanted with the same tumour generation. In each chemotherapy trial drug(s) was administered weekly X 4, beginning on week 5 following tumour transplantation. Animals were sacrificed 9 weeks after implantation. Control animals at this time demonstrated diffuse metastatic pertioneal seeding with tumour invasion into adjacent organs. Pulmonary metastases were rare. At the time of sacrifice all visible tumour was carefully removed and the total wet tumour weight from each animal was recorded. Effectiveness of chemotherapy was judged by comparing the percentage of animals having tumour and by comparing the to-

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Table 1. Flank nodule model

Treatment	% Animals with tumour present at 4 weeks	WT. of tumour in grams at 4 weeks
Control		
Saline (0.5 ml/wk 1, 2, 3)	93% (14/15)	4.08 ± 1.04
Neocarcinostatin (1,000 u/kg wk 1, 2, 3)	100% (10/10)	6.6 grams
Neocarcinostatin (2,000 u/kg wk 1, 2, 3)	71% (5/7)	1.60 ± 0.70 > 0.05**
Chrlorozotocin (40 mg/kg wk 1, 2)	66% (2/3)	3.63 grams
Bleomycin (15 u/kg wk 1, 2, 3)	100% (10/10)	3.30 ± 0.67
Vindesine (1 mg/kg wk 1, 2)	12% (1/8) < 0.025*	0.1 mg
Maytansine (0.25 mg/kg wk 1; 0.175 mg/kg wk 3)	100% (11/11)	1.03 ± 0.31 < 0.005**
Carminomycin (0.5 mg/kg wk 1, 2, 3)	100% (10/10)	2.53 ± 0.66
Carminomycin (1 mg/kg wk 1, 2, 3)	100% (6/6)	2.85 ± 0.40

* Chi sq analysis
** Student analysis
*** Hydroxyurea 100 mg/kg. Three times weekly for three weeks

Table 2. Metastatic model. Treated 5 weeks after tumour implanation sac 9 weeks after tumour implanation

Treatment	% Animals with tumour present at sac	WT. of tumour in gram present at sac *P value
Control		
Saline (0.5 ml/wk 5, 6, 7 8)	83% (10/12)	78.0 ± 46.0
VDR (wk 5, 6, 7, 8)	90% (9/10)	4.6 ± 0.9
VDR (wk 5 & 7) + CTX (wk 6 & 8)	100% (10/10)	5.8 ± 2.2 > 0.05**
VDR (wk 5 & 7) + VDR & CTX (wk 6 & 8)	90% 9/10)	8.4 ± 4.0
VDR (wk 5 & 7) + CIS & CTX (wk 6 & 8)	20% (2/10) < 0.025*	21.5 ± 7.5
Control	84% (18/24)	93.3 ± 15.9
VDR (wk 5 & 7) + CIS & CTX (wk 6 & 8)	76% (10/13)	9.0 ± 2.5 < 0.001**
Control	84% (16/20)	56.4 ± 8.8
VDR (wk (5 & 7) + CIS & CTX (wk 6 & 8)	61% (16/30)	8.9 ± 0.9 < 0.001**

* Chi sq analysis
** Student analysis
Vindesine (VDR) 0.5 mg/kg; Cyclophosphamide (CTX) 40 mg/kg; Cisplatin (CIS) 5 mg/kg

tal weight of tumour at the time of sacrifice with that of controls. Agents tested in the metastatic disease model included vindesin (VDR) 0.5 mg/kg, alone, in combination with cyclophosphamide (CTX), 40 mg/kg or in combination with CTX and cisplatin II (CIS), 5 mg/kg.

Results

The only single agent in these experiments that effectively reduced the incidence and weight of tumour present at 4 weeks in the nodule transplant model was VDR ($P \leqslant 0.025$). While maytansine did not reduce the tumour incidence at 4 weeks, there was a significant reduction in wet tumour weight in animals treated with this compound ($P < 0.005$). Neocarcinostatin, with the higher dosage considerably reduced tumour weight though it did not reach significance ($P < 0.05$). Carminomycin, chlorozotocin and bleomycin, at the dosage schedules employed, were ineffective (Table 1).

Table 2 shows the results of treatment in the metastatic transplant model. Initial studies with a combined treatment regimen with VDR, CTX and CIS demonstrated a reduction in tumour incidence to 20% ($P < 0.025$). With additional studies of this combination, the statistical significance of a decrease in tumour incidence with this drug combination was lost. However, there were still 25 of 53 animals (47.2%) that had no tumour found at the time of sacrifice.

When the weight of the tumour present at sacrifice was analysed in the first group of animals (Table 2) it was found that vindesin alone, or in combination greatly reduced tumour weight. This reduction did not reach statistical significance due to the very large standard deviation noted in the control group of animals bearing this tumour generation (78.8 ± 46 g). The average weight of tumour in each group was calculated by employing as a denominator the number of animals with tumour and not the total number of animals in each group.

The remaining two groups in Table 2 represent two repetitions of VDR, CTX and CIS treatment regimen. On these occasions tumour generations grew more uniformly in the control animals. The reduction in tumour weight employing the 3 drug protocol was statistically significant in each experiment ($P < 0.001$).

Discussion

The search for effective chemotherapy for patients with renal carcinoma has been singularly diappointing. In 1977, Hrushesky and Murphy reviewed the status of chemotherapy in the treatment of metastatic renal cancer [5]. Of the single agents tested, the most successful was vinblastine (33% objective response rate). The response achieved with 5-fluorouracil (5-FU) or hydroxyurea (HU) was 5%, while methyl CCNU (1-(2-chlorethyl)-3-(4-methyl cyclohexy 1)-1-nitrosourea) showed a 7% response rate. Various combinations of these and other agents have resulted in reported responses seen in only 5% of patients and addition of endocrine therapy does not apparently improve overall chemotherapeutic response. Todd, utilizing methyl-GAG (methylglyoxal-bis-guanylhydrozone) reported 3 partial and one complete response in 18 patients; however the mean duration of response was only 7.5 weeks [15].

More recently various investigators have studied combinations of agent in the management of patients with metastatic renal carcinoma. Baumgartner, using a combination of methotrexate (with citrovorum factor rescue),vincristine, bleomycin and CTX or peptichemio, reported objective partial remissions in 5 of 12 patients with this neoplasm [1]. Utilizing a combination of vinblastine, methotrexate and bleomycin (with or without tamoxifen) in 28 patients, Levi reported a 36% partial response rate [6]. Treatment of 40 patients with adriamycin and CTX resulted in 8 partial remissions in Miller's series [7]. Richards administered CCNU (1-(2-chloroethyl)-3-cyclohexyl-1 nitrosourea) along with bleomycin, adriamycin and methylprednisolone in 14 patients, demonstrating 3 partial remissions lasting 3, 3 and 18 months, respectively [9]. Finally, Tally reported on 32 patients treated with CTX, vinblastine, hydroxyurea and prednisone, demonstrating 1 complete and 6 partial remissions [14]. Mean survival time of the patients experiencing partial remission was 18 months versus 5 months in the non-responders.

The search for effective chemotherapy for the treatment of patients with metastatic renal carcinoma must continue and this need has stimulated a number of chemotherapeutic trials in murine models of this disease. Soloway studied a renal cortical carcinoma (MKT-Cdc) arising spontaneously in a male BALB/Cd mouse and maintained in BALB/cAnN female mice. He reported that diethylstilbestrol (DES) and testosterone retarded tumour growth while medroxyprogesterone (Provera) was ineffective [12].Murphy and Hrushesky studied the same renal carcinoma maintained in BALB/c/Cd male mice. Unlike Soloway, these workers found that tumour growth was increased with testosterone and DES, while again, medroxyprogesterone was ineffective [8]. These investigators also reported that vinblastine and CCNU demonstrated anti-tumour activity in this animal model [4]. Shefner and Marlow further studied the same tumour and found that bleomycin, hydroxyurea and CTX were ineffective agents, whereas adriamycin and vinblastine increased the mean survival time by greater than 25%. Two nitrosoureas (methyl-CCNU and BCNU) were highly effective in reducing tumour size and prolonged survival by greater than 25% [11].

Against this background, we began chemotherapeutic trials in a spontaneously arising renal carcinoma in a male Wistar-Lewis rat. As we studied this tumour further, we found that it was nearly ideal in its characteristics, compared to the same carcinoma in the human. This tumour 1) arose spontaneously; 2) histologically proved to be a true carcinoma; 3) was endocrine independent; 4) was easily transplantable and 5) had a predictable growth rate. The fact that the tumour was not hormonally independent was established by transplantation to female Wistar-Lewis rats, normal and castrated males, as well as normal males treated with DES, progesterone and testosterone. The tumour was also assayed for androgen, estrogen and progesterone receptors with none being found [2].

Our studies were carried out in animals receiving transplants of this tumour in one of two fashions. A flank nodule was easily achieved by surgical transplantation. However the tumour did not metastasise from this site, so that mean survival time was an unpredictable indication of the effect of tumour on the host. Furthermore, the tumour nodule would outgrow its blood supply after a two month interval with resultant central necrosis, rendering tri-dimensional tumour treatment (beyond 8 weeks) an unreliable indicator of anti-neoplastic effect of chemotherapeutic trials. For these reasons, the nodule model served only as a method of screening various chemotherapeutic compounds for effectiveness against the early growth of the nodule. Agents so identified would be tested alone and in combination against a metastatic disease model, produced by intraperitoneal transplantation of tumour to a previously splenectomised animal.

We have previously reported the results of a number of chemotherapeutic trials in these tumour models [2, 3]. In the nodule model, 5-FU (30 mg/kg weekly X 3), hydroxyurea (100 mg/kg 3 times weekly X 3) and streptozotocin (100 mg/kg in a single dose) failed to influence the growth of the tumour. Adriamycin (5 mg/kg weekly X 2) and vinblastine (0.3 mg/kg weekly X 2) resulted in a 50% decrease in tumour incidence, although this reduction was not statistically significant. On the other hand, CTX (40 mg/kg weekly X 3) eradicated 100% of tumours if started one week post-implantation ($P < 0.005$ and $P < 0.001$, in two separate experiments). If this agent was started at 2 weeks, rather than one week following implantation it also showed considerable anti-tumour activity. In fact, this agent continued to show anti-tumour activity even if therapy was not initiated until 5 weeks following implantation [2]. Cisplatin

has been ineffective in clinical trials in patients with renal cancer [10]. When used alone, it was similarly ineffective in this murine model [2]. At dosages of 3 mg/kg, 5 mg/kg and 8.5 mg/kg it failed to alter tumour growth; increasing dosage merely causing an increased animal mortality. However, it was noted that if CIS was given 1 week after tumour transplantation and followed by CTX on week 2 and week 3, all tumour was eradicated. If CTX alone was given on week 2 and week 3 (not preceded by CIS on week 1) 50% of animals still had tumour growth at 4 weeks. This apparent additive effect between CIS and CTX has been borne out in repeated studies in both the nodule and metastatic model [2, 3].

The present experiment showed that carminomycin, chlorozotocin and bleomycin in the dosages tested demonstrated no activity on tumour growth in this neoplasm. Maytansine and neocarzinostatin (the latter at the higher dosage regimen), resulted in a considerable decrease in wet tumour weight at 4 weeks which in the case of maytansine reached significance ($P < 0.005$).

The observation that vinblastine resulted in a 50% reduction in tumour incidence at 4 weeks led to the testing of another vinca alkaloid, vindesin (VDR). VDR was effective in eradicating virtually all tumour if initiated one week following implantation ($P < 0.025$). The administration of this agent at levels above 1 mg/kg resulted in unacceptable toxicity. For all combination agent studies, the dosage of this agent was therefore decreased to 0.5 mg/kg.

The results achieved when CTX was used alone or in combination with CIS, adriamycin or vinblastine in the metastatic disease model have been previously reported [2]. Reduction in the amount of tumour present at sacrifice was seen in all combinations tested. CTX alone reduced tumour to one-third the size of the control group ($P < 0.05$). The addition of CIS to CTX resulted in further reduction of tumour weight ($P < 0.001$). The most effective means of combining CTX and CIS was found to be achieved by delivering both agents together on weeks 5 and 7. There was nearly a 75% reduction in final tumour weight ($P < 0.001$). Alternatively, vinblastine and adriamycin in combination with CTX were less effective regimens against the neoplasm. The present study showed even more impressive reductions of tumour weight achieved with CTX along with CIS and VIN. This observation leads us to the conclusion that a combined treatment protocol consisting of these three compounds might be effective in patients with metastatic renal carcinoma. A treatment protocol has recently been established for the evaluation of this regimen in such patients.

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