Cancer Chemother Pharmacol (1992) 30: 70–72

Cancer Chemotherapy and Pharmacology

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Short communication

A new liver-tumor model in the rat*

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Received 15 July 1991/Accepted 12 December 1991

Summary. A new tumor model for testing and comparing the effect of different forms of treatment on liver tumors is described. Single tumors were induced in the liver of rats by the implantation of small pieces taken from a subcutaneously growing tumor. Tumor growth was determined by measuring the weight of the implanted tumors after the animals had been killed. In this way, weight curves for treated and untreated tumors could be defined. The weight of untreated liver tumors increased exponentially with time (tumor weight in milligrams = $1 + e^{(t-0.31)/5}$). In addition, tumor growth defined as the geometric mean of three perpendicular diameters was determined. Tumor-diameter curves showed a linear increase with time in the untreated groups (tumor diameter in millimeters = $0.4\,t + 1.90$). The model was tested by assessing the effect of intraperitoneally injected cisplatin. The dose chosen produced a marked delay in tumor growth. On the basis of the weight gain shown by the treated animals and tumor growth delay, a therapeutic index can be defined, thus enabling to compare quantitatively different forms of treatment according to their antitumor effect and toxicity.

Introduction

Cancer metastasis to the liver is generally considered to be incurable. Numerous different modalities of treatment have not yet improved this situation [3]. Unfortunately, no animal model is available that enables the quantitative measurement of the effect of a given form of chemotherapy on liver tumors according to its antitumor activity and toxicity. In current animal models using liver tumors, tumor-cell suspensions are injected into either the portal vein [7] or a mesenteric vein [2], leading to multiple tumors that vary considerably in number and size. In contrast, in the model described in the present report, a piece of a single tumor is implanted directly into the liver of each experimental animal. Tumor growth is reflected by the increase in tumor weight and tumor diameter. The toxicity of a given treatment corresponds to the changes in body weight shown by the animals.

A therapeutic index [4, 6] can be defined as the ratio of the delay in tumor growth (tumor weight) to the weight loss displayed by the animals, thus enabling the comparison of different forms of treatment, e.g., drug a (dose x, i.v.) to drug b (dose y, i.p.). In addition, tumor growth defined as the geometric mean of three perpendicular diameters was determined. This report describes the methods used to induce and measure single liver tumors in rats and – as an example – the response of these lesions to the i.p. injection of cisplatin into tumor-bearing animals.

Materials and methods

Experimental animals and husbandry. SPF Wag/Rij rats aged 3 months and weighing 270–290 g were used. Animals were acclimatized for at least 3 weeks in rooms maintained under controlled conditions (artificial lighting from 0700 to 1900 hours; ventilation, 15 air changes/h; temperature 22° C; relative humidity, 55%). Rats were housed in polycarbonate cages (Makrolon type III, three rats per cage) on presterilized wood shavings and were given Hope Farm AM II food (Woerden, The Netherlands) and plain tap water ad libitum.

Tumor model. Colon adenocarcinoma CC 531 [5], a moderately differentiated and syngeneic tumor that is transplantable into Wag/Rij rats, was maintained s.c. On the day of inoculation, the tumor was excised, cut into 1-mm^3 pieces, and kept in phosphate-buffered saline solution. After a small midline laparotomy had been performed with the rats under ether anesthesia, an incision (maximally 2 mm long and 2 mm deep) was made in the median lobe of the liver and a piece of tumor was implanted and covered with Lyostypt (B. Braun Melsungen AG, FRG).

At the end of the 35-day observation period, the animals were killed by exsanguination. Each tumor was carefully dissected under an operation microscope, after which all remaining liver tissue was removed with

* This work was supported in part by the European Community Concerted Action Programme on Drug Carriers in Cancer Chemotherapy and in part by Bristol Myers, Germany

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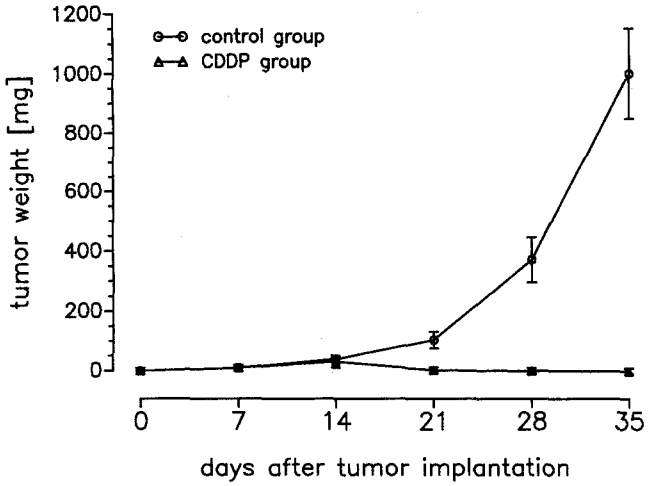

Fig. 1. Tumor-weight curve for CC531 tumors grown intrahepatically in untreated controls and in rats treated i.p. with 4 mg/kg cisplatin (*CDDP*) on day 10 after tumor implantation

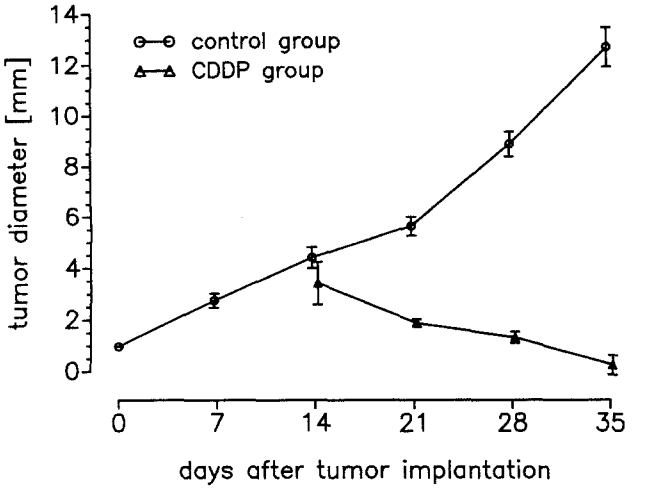

Fig. 2. Tumor-diameter curves (plotted as means and standard deviations of the geometric means of three perpendicular diameters) for CC531 tumors grown intrahepatically in untreated controls and in rats treated i.p. with 4 mg/kg cisplatin (*CDDP*) on day 10 after tumor implantation

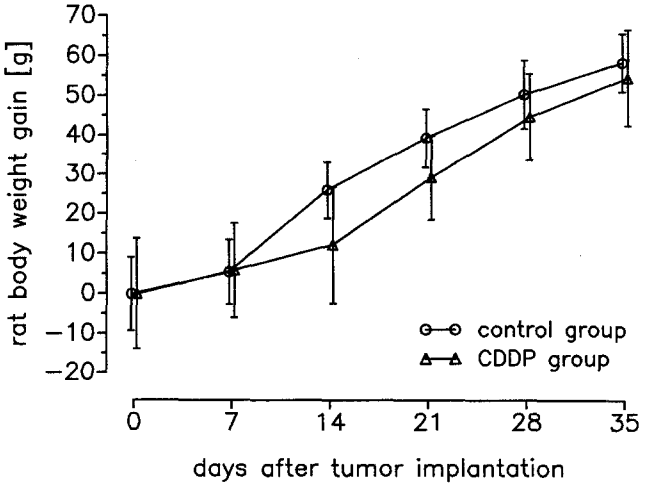

Fig. 3. Change in the body weight of untreated controls and CDDP-treated rats as expressed in grams

microsurgical scissors. The dimensions of the tumor were determined using digital calipers (Mitutoyo, Japan) by taking the largest width, length, and height as diameters. From these three values, the geometric mean was calculated and the values were expressed in tenths of millimeters. Thereafter, the weight of the tumor in milligrams was determined using a Mettler PK300 precision balance (Mettler, FRG). The histology and pathology of the implanted liver tumour in the presence and absence of laser treatment have been described elsewhere [8, 9].

Experimental design. Rats were randomly divided into nine groups, each group containing five rats. Tumors of untreated rats were measured at weeks 1–5 in one group per week. Tumors of treated rats were measured at weeks 2–5, again in one group each week.

Treatment and weighing. At day 10 after tumor implantation 20 tumor-bearing rats received an i.p. injection of 4 mg/kg cisplatin (0.5 mg/ml; Bristol Myers, Weesp, The Netherlands). Body weight was assessed every week during the period of observation.

Calculations and statistics. Computer-based curve fitting was performed to describe the tumor weight × time curves. Simple linear regression was used to determine the increase in tumor diameters between day 14 and day 35.

Results

All tumor-implanted rats could be used for the experiments. Complications from the operations were not observed. The untreated rats showed an exponential increase in tumor weight (Fig. 1). The relationship of tumor weight with time could be described by the equation $y = U_{ab}+e^{(t-Td)/T}$ using the calculated values T = 5.0 days, Td = 0.31 days, and Uab = 1.0 mg, where *y* represents the weight of the tumors in milligrams; *Uab*, the size of the tumors at the time of implantation (day 0); *t*, the time in days; *Td*, the time delay (in days) until the beginning of exponential growth; and *T*, the time constant in days. From this data, a weight-doubling time of approximately 3.5 days could be calculated.

The corresponding sizes of the tumors, expressed as the geometric means of three perpendicular diameters, showed a linear increase in the untreated groups (Fig. 2). For the period of day 14 to day 35, a regression was defined that could be described by the equation y = 0.40 t – 1.90 (untreated group), where *y* represents the size of the liver tumors in millimeters and *t*, the time in days. The goodness of fit, represented by correlation coefficient (multipler) was 0.9676/0.9827 (control group, a/b).

The cisplatin-treated groups showed no increase in tumor weight or size during the 35-day observation period [tumor diameter in millimeters, –0.14 t + 5.25; multiple *r*, 0.9241/0.9546 (CDDP group, a/b), Figs. 1, 2]. The growth of the liver tumors was slower (0.40 mm/day) than that previously reported for s.c. tumors (0.54 mm/day) and faster than that observed for mesenteric tumors (0.38 mm/day) of the same histology [6].

Indications of toxicity were given by changes in the body weight of the animals (Fig. 3). Both the untreated and the cisplatin-treated groups showed an increase in body weight over the 35-day period. However, the cisplatin-treated rats exhibited less weight gain than did the untreated animals, as would be expected due to the cytotoxicity of the drug.

Discussion

The present rat model enables the quantitative assessment of the effect of treatment modalities on tumor growth in the liver. The model was set up to simulate hepatic metastases as part of a European Community Concerted Action Programme on the treatment of colon cancer.

The prior development of an i.p. tumor model gave us valuable experience that was applied in the design of the model described herein [4, 6]. Both models involved only one tumor per animal, and the growth of the lesion could be calculated by determining its dimensions. However, there was one important difference: in the previous i.p. model, subsequent tumor measurements was carried out in the same animals, which necessitated repeat laparatomies, whereas in the present model, tumor growth was assessed in different animals that had been sequentially killed. The advantages of the latter situation were:

1. It required no relaparatomy, which can be a stressful procedure that may produce alterations in tumor growth [6].

2. The dimensions could be measured more accurately after the tumor had been taken out.

3. The more valuable parameter, tumor weight, could be established. The important disadvantage of the new model was that it required a larger number of animals.

The parameter tumor weight provides a more accurate measure of tumor growth than does the geometric mean of three perpendicular diameters [1]. This was reflected by the growth curves obtained in our study. The tumor-weight curves showed an exponential increase, whereas the diameters of the tumors increased linearly. Reasonable regression curves for the tumor diameters could be defined only for the period between day 14 and day 35. This would suggest that the accuracy of the previous i.p. model might have been improved if tumor weight instead of tumor diameter had been used as the parameter for tumor growth.

Concurrent measurements of tumor growth and animal body weight enables the estimation of the therapeutic index for a given treatment as described in the i.p. model [4, 6]. Therapeutic index can be defined as the ratio of tumor-growth delay to weight loss. Tumor-growth delay represents the time in days required for the lesion to grow to a predetermined, arbitrarily chosen size. Weight loss can be calculated from the area under the body weight (in grams) $\times$ time (in days) curve obtained for the control groups minus that found for the treated groups. Thus, different forms of treatment can be readily compared. However, the use of tumor-growth and animal-weight measurements to provide a therapeutic index is only reliable if precise standardization of the preparation of the tumor implants and the implantation procedure is included. A therapeutic index was not determined in the present study because no tumor regrowth occurred during the observation period.

Comparison of the data obtained for the i.p. model with those gathered using the liver-tumor model revealed that the liver tumors impaired the condition of the animals to a lesser extent than did the mesenteric tumors. In the present study untreated rats gained about 60 g (vs 55 g in the CDDP treated groups) over a period of 35 days, whereas untreated rats in the i.p. model gained only about 35 g (vs no weight gain in a group treated i.p. with 4 mg/kg cisplatin) over the same period. Furthermore, the tumors in the liver seemed to be more susceptiple to i.p. cisplatin than were the mesenteric tumors. In the i.p. model, tumor regrowth occurred after day 17, whereas no regrowth was observed in the present model throughout the 35-day observation period. Although these discrepancies might be attributable to different tumor vascularization, we have not yet found a definitive explanation for them.

Acknowledgements. We would like to thank Mr. A. Birke for calculating the statistics.

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