A new intraperitoneal tumor model in the rat

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Summary. A new tumour model that is particularly suitable for testing intraperitoneal chemotherapy is described. Single tumours were induced to grow in the mesentery of rats by the implantation of small pieces taken from subcutaneous tumours. Tumour growth was monitored by repeated laparotomies at which the tumour size was measured with calipers. In this way, growth curves of treated and untreated tumours could be defined. The diameter of untreated intraperitoneal tumours increased linearly with time [diameter (mm) = 0.39 t (days) +2.4]. Tests using different numbers of laparotomies showed that the procedure itself had little influence on growth. Cell kinetic studies of 6-mm tumours showed a mean labelling index of 31% and a volume-doubling time of 3.9 days, resulting in cell-loss factors probably in excess of 70%. The model was tested by assessing the effect of the chemotherapeutic agent cisplatin. Regression and regrowth could be satisfactorily followed, leading to estimates of growth delay. This model therefore provides a quantitative way to assess the response of intraperitoneal tumours to chemotherapy.

Introduction

Intraperitoneal chemotherapy for cancers restricted to the peritoneal cavity has demonstrated pharmacokinetic advantages over intravenous therapy, both in experimental animals and in the clinic [3]. There is a need, however, for suitable animal models that measure the effectiveness of different forms of therapy. One relevant and quantitative parameter for measuring therapeutic effectiveness is the delay in tumor growth [1, 10]. Commonly used rat models are those of intraperitoneal metastases after intraperitoneal injection of a tumor-cell suspension [5, 6]. Such models are not suitable for measuring the delay in tumor growth, however, because they provide multiple metastases of various sizes and at different, often unmeasurable, sites. The new model described provides a single intraperitoneal tumor that is located at a defined site in the mesentery. The antitumor effect of different forms of treatment can be readily compared by measuring the growth delay of the tumor that is assessable by repeat laparotomy. One index of treatment-induced toxicity, as with other tumor models, can be measured concurrently by monitoring body-weight changes.

This paper reports on the methods for inducing and monitoring the tumours, their cell kinetic properties and their responses to an intraperitoneally injected drug.

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 with controlled conditions (artificial lighting from 0700 to 1900 hours; ventilation: 15 air changes/h, 22°C and relative humidity of 55%). Rats were housed in polycarbonate cages (Makrolon type III, three rats per cage) on presterilized wood shavings. They were given Hope Farm AMII food (Woerden, The Netherlands) and plain tap water ad libitum.

Tumor model. The CC531 tumor, a moderately differentiated adenocarcinoma of the rat colon, was used throughout the study. The tumour was originally induced by six weekly injections of 30 mg/kg 1,2-dimethylhydrazine (DMH) into a Wag/Rij rat. It arose in the proximal colon 40 weeks later [7] and was kept in culture flasks in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum (FCS). A single-cell suspension was prepared by trypsinization, counting in a haemocytometer, centrifuging (1,000 rpm, 5 min) and suspending in phosphate-buffered saline (2 × 106 cells/ml). All cell suspensions contained over 95% viable cells as assessed by trypan blue exclusion.

Preparation of tumor pieces for implant. A tumor-cell suspension (1 ml) containing 2 × 106 cells was injected into the flank of the rats. After 3 weeks a solid tumor with a diameter of approximately 3 cm was produced. The tumor was excised and cut into slices of 1.5 mm. For this purpose, a custom-made tool consisting of five razor blades separated by a constant distance of 1.5 mm was used. The tumor slices showed two different regions: a necrotic centre and a solid, viable outer border. From the grossly viable part, cylinders of 3-mm diameter were punched. Each

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tumour piece therefore measured 3 mm in diameter by 1.5 mm in thickness. The tumour pieces were put in physiological saline and were kept for a maximum of 2 h at room temperature before implantation. For the subcutaneous tumours used for chemosensitivity testing, cells were injected as described above and the rats were treated when the tumours had reached a mean diameter of between 6 and 10 mm.

**Intraperitoneal implantation.** Rats were anaesthetized with ether. After shaving and preparation of the operation site with 70% ethanol, a 1-cm incision was made into the median ventral abdominal wall, close to the transitions of the upper and lower quadrants. Part of the small intestine was exposed and the tumour pieces were implanted by a haemoclip fixed to the distal part of the mesentery. Care was taken not to occlude the respective mesenteric vein. The bowel was repositioned and the abdomen, closed by 3–4 simple interrupted sutures.

**Intraperitoneal tumour characterisation.** Solid intraperitoneal tumours were not necrotic during the period of observation (63 days). However, large (approximately 2 cm) tumours began to develop small necrotic centres. This tendency was significantly lower in comparison with subcutaneously growing tumours. Tumour pieces were implanted in highly vascularized locations, resulting in ingrowing of blood vessels from the mesentery into the tumour. As the tumours grew, they were covered by connective tissue containing a tight network of blood vessels. This relatively rich vascular supply is probably the reason why necrotic centres occurred only in large intraperitoneal tumours. Metastases were not detected during the observation period of 63 days.

**Tumour measurements.** A laparotomy was performed once every 2 weeks to measure the intraperitoneally growing tumour. The rats were anaesthetized with ether and, after the small intestine had been exposed, tumour size was assessed by measuring the three perpendicular diameters of the tumour with digital calipers. The geometric mean of the three values was then calculated [1]. The bowel was repositioned and the abdomen, closed as described above. Depending on the experimental group, each rat received several laparotomies. It was therefore necessary to guard against possible infections. Sterilization of the surgical instruments was routine, and Betadine solution was used for disinfecting the skin. In addition, every unnecessary irritation of the bowel was avoided, since a mechanical ileus could have been caused. For subcutaneous tumours, three perpendicular diameters were measured using calipers without the need for an operation or anaesthetic.

**Cell kinetics.** The cell kinetic parameters of intraperitoneal CC531 tumours were measured using the thymidine analogue 5'-iododeoxyuridine (IUdR) and flow cytometry. Rats were injected intraperitoneally with 30 mg/kg IUdR and killed at intervals up to 7 h, at which time the tumours were excised. The methods of preparation and analysis have been described elsewhere [2]. Briefly, tumour pieces fixed in 70% ethanol were treated with pepsin to produce suspensions of nuclei. DNA was partially denatured with 2 N HCl and nuclei were stained with an IUdR-DNA-specific mouse monoclonal antibody (Partec AG, Switzerland) followed by a fluorescein isothiocyanate (FITC)-conjugated antimouse antibody. Nuclei were subsequently counter-stained with propidium iodide for total DNA content and then analyzed by flow cytometry for red and green fluorescence simultaneously. Windows placed around selected subpopulations were used to calculate the labelling index (percentage of green fluorescent cells) and the position of the labelled (green) cells relative to G1 and G2 so as to estimate the length of the DNA synthesis phase [2]. Flow cytometry was carried out using a FACScan (Becton Dickinson; Sunnyvale, Calif.).

**Results**

Four of the initial attempts to implant tumour pieces in the mesentery proved to be technical failures, but after we had familiarized ourselves with the technique, all operated rats could be used for the experiments. The rats used for tumour-growth tests were divided into four groups. Animals in groups A, B, and C were not treated, whereas those in group D received 4 mg/kg cisplatin intraperitoneally on the 10th day after implantation. Groups A and B were used to determine growth in untreated rats. As we did not know whether the animals could survive a laparotomy every week, we decided to perform the operation every 2 weeks. To ensure sufficient measurements, the laparotomies were done on alternate weeks for groups A and B, respectively (see Fig. 1). After the implant operation, tumours were measured separately for each rat, on days 52 and 59, although the differences were not statistically significant (Fig. 1). The relationship of tumour diameter with time could be described by the equation:

\[ y = 0.39t + 2.4, \]

where \( y \) represents the size of the mesenteric tumour in millimeters and \( t \) represents the number of days. There was no difference in tumour-growth rate between groups A and B. The tumours in group C were slightly smaller when measured on days 52 and 59, although the differences were small and the growth rate appeared to be identical. This indicated that the number of operations performed in groups A and B had little influence on tumour growth. The cisplatin-treated group showed a marked delay in growth...