

A New Peritoneal Carcinomatosis Model in Cyclosporine Immunosuppressed Rats

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Abstract. Colorectal cancer (CRC) cells (HT29) were injected into the intraperitoneal (*i.p.*) cavity of immunosuppressed male Sprague Dawley rats. Immunosuppression was achieved via a daily *i.p.* injection of cyclosporine 35 mg/kg/day. Three groups of rats ($n=6$ per group) were injected with 20, 50 or 100 million HT29 cells. All animals developed tumours indicating that this animal model may prove useful for future research in human colorectal carcinomatosis.

The peritoneum is a common site of metastatic or recurrent tumour growth in patients with colorectal cancer (CRC). Ten to twenty percent of gastrointestinal cancer patients are affected by peritoneal carcinomatosis with survival below 6 months (1). The conventional treatment regimes of complete resection of all macroscopic disease and adjuvant intraperitoneal (*i.p.*) chemotherapy used to treat this disease have not proved particularly effective and there is a need to develop new treatments to target peritoneal carcinomatosis (1, 2). The development of heated intra-abdominal chemotherapy (HIIC) has been reported to achieve 5-year survival rates above 30% (3). However, further improvement in this type of chemotherapy is hindered due to the lack of appropriate experimental animal models of human colorectal carcinomatosis which are both accurate in their pathophysiological behaviour and economically viable.

A few nude mice colorectal carcinomatosis models have been described in the literature (4,5). Unfortunately, nude mice models are both expensive and may not accurately reflect disease progression and tumour pathophysiological behaviour compared to that in immunocompetent animals. For example, current nude mice models of peritoneal

carcinomatosis do not allow a large tumour burden or ascites formation, aspects which more closely mimic the human disease.

Steel and Peckman have proposed that immunocompetent animals treated with the appropriate drugs could bear human carcinomas with minimum immunosuppression (6) and indeed, it has been reported that human breast and melanoma cells were able to grow in immunosuppressed rats (7-9). Using a larger animal such as a rat, rather than mice, may thus increase the relevance to clinical practice as well as simplify the overall handling and logistics of surgical procedures. A number of peritoneal carcinomatosis studies using a syngeneic rat model have been reported (10, 11). However, the natural history of carcinomatosis induced with rat syngeneic tumour cells may be quite different to that of human cancer cells. The larger animal size and longer natural lifespan of rats have several advantages over nude mice models, including the possibility of surgical procedures, repeated blood sampling and carrying a much greater tumour burden. A cyclosporine immunosuppressed rat carcinomatosis model was therefore developed in this study. Cyclosporine is a polypeptide derived from the fungus *Tolypocaladium inflatum* Gams. It has been suggested that cyclosporine acts mainly by suppressing the release of interleukin-1 from macrophages, necessary for the activation of T-lymphocytes. It also inhibits the release of interleukin-2 which is necessary for the proliferation of activated T lymphocytes (12). Cyclosporine side effects include renal toxicity, weight loss, hepatotoxicity, lymphopenia, and an increased risk of opportunistic infection.

Here we report the results of a cyclosporine immunosuppressed rat peritoneal carcinomatosis model using the human colorectal cancer (CRC) cell line HT29. To the best of our knowledge, this model is unique.

Materials and Methods

Animals. Male Sprague Dawley rats ($n=18$) weighing between 200 and 250 g were purchased from the Biological Resources Centre of University of New South Wales and housed at the Commonwealth

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Scientific and Industrial Research Organisation (CSIRO) animal facility, North Ryde, Australia. This study was approved by the CSIRO animal ethics committee (approval no. 06/06). The animals were kept under specific-pathogen free conditions with free access to autoclaved food and water.

Cyclosporine injections. Forty-eight hours before tumour cell implantation, cyclosporine (Sandimum 50mg/ml, Novartis, North Ryde, NSW, Australia) was given *i.p.* at a concentration of 35 mg/kg/day. Thereafter, the same dose was given *i.p.* daily. Blood samples from rat tails were collected in EDTA microtainer (Becton Dickinson, North Ryde, NSW, Australia) at 0 h, 48 h and then on a weekly basis. Blood trough levels of cyclosporine were analysed at South East Area Laboratory Service (SEALS) Pathology by the fluorescence polarization immunoassay (FPIA) method. Cyclosporine was measured using a CEDIA Cyclosporine Plus Assay (Microgenics, Beckman Coulter, Gladesville, NSW, Australia) performed on a Beckman Coulter DXC analyzer.

Tumour cell line preparation. The colorectal cancer cell line HT29 was obtained from the American Type Culture Collection and maintained in RPMI-1640 medium (Invitrogen, Mount Waverley, VIC, Australia) supplemented with 10% heat-inactivated foetal calf serum, 1% penicillin-streptomycin and 200 mM glutamine (Invitrogen). The cells were grown in 75 cm² flasks at 37°C in a 5% CO₂ humidified atmosphere and routinely checked for mycoplasma contamination. Semi-confluent cells were trypsinized by using 0.25% trypsin (Invitrogen) for 10 min and then centrifuged at 1500 rpm and resuspended in Dulbecco's phosphate-buffered saline (PBS) (Invitrogen). The cells were counted using a Neubaur chamber (Lombs Scientific, Taren Point, NSW, Australia) and viability determined by trypan blue exclusion test. Viable cells were washed once in PBS and resuspended in the same buffer for implantation.

Tumour implantation. The rats were randomized into one of three treatment groups (n=6 per treatment) and lightly anaesthetized with fluothane. Each rat in a group received a total volume of 2 ml containing a known number of HT29 cells resuspended in PBS, in Group 1 20 million HT29 cells, Group 2, 50 million HT29 cells and Group 3, 100 million HT29 cells. After injection, the rats were transferred to their respective cages and monitored until fully recovered (usually within 5 minutes). The animals were monitored daily and their weight recorded every three days. At the end of the experiment (49 days) the animals were scarified by CO₂ inhalation and the peritoneal cavity examined for tumour growth. All the tumours were excised, fixed in 10% formalin and paraffin embedded (Lombs Scientific) for further analysis.

Histological study. Tumour samples were cut into 5 micron sections and stained with hematoxylin and eosin (H&E) as per the protocol of SEALS Pathology, Australia. The slices were photodocumented by conventional optical microscopy (Leica DMLB, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a digital camera (Leica DC 200).

Results

Peritoneal tumour growth. All the animals developed visible peritoneal nodules, without forming any ascites. The mean total number of tumour nodules was 5 (range 1-11), 15

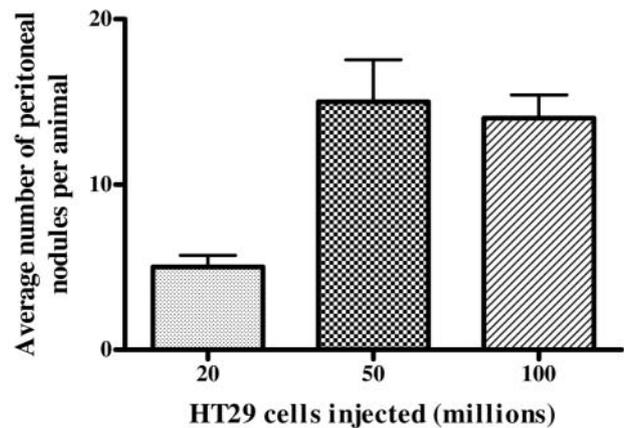


Figure 1. Peritoneal tumours in immunosuppressed rats. Rats were injected intraperitoneally with 20, 50, 100 million HT29 cells (n=6 per group).

(range 9-22) and 14 (range 5-20) in groups 1-3, respectively (Figure 1). Tumour distribution in the peritoneal cavity was widespread and included the peritoneum, mesentery, omentum, illeocecum, liver, kidney and spleen (Figure 2). A substantial proportion of the tumours were located in the mesentery (51%, 20% and 42% in the 20, 50 and 100 million groups, respectively). The HT29 tumour was quite aggressive and in most rats it penetrated the mucosa. In six rats (four in group 3 and two in group 2), tumours invaded the mucosa thereby blocking the lower intestine. This caused diarrhoea and weight loss in these rats. These rats were sacrificed earlier than the planned end of the experiment. No other adverse effects were observed in the remaining rats.

Blood cyclosporine levels. After 48 h, the plasma mean trough cyclosporine level was 2883 ng/ml. All immunosuppressed animals showed moderate to high trough levels of cyclosporine and at the end of the experiment, the mean cyclosporine level was 7388±2093 ng/ml. No rats showed any sign of toxicity or premature death due to the cyclosporine. The animals gained weight during the course of the experiment except for the 6 rats which were culled due to tumour burden.

Histology. Figure 3 shows invasion of the rat mucosa by moderately differentiated adenocarcinoma cells. The tumour cells appeared anaplastic with enlarged, irregular nuclei and an increased nuclear/cytoplasmic ratio. The overall mitotic index was very high.

Discussion

In the past, Hoogenhout *et al.* used a combination of cyclophosphamide and cyclosporin A in addition to total

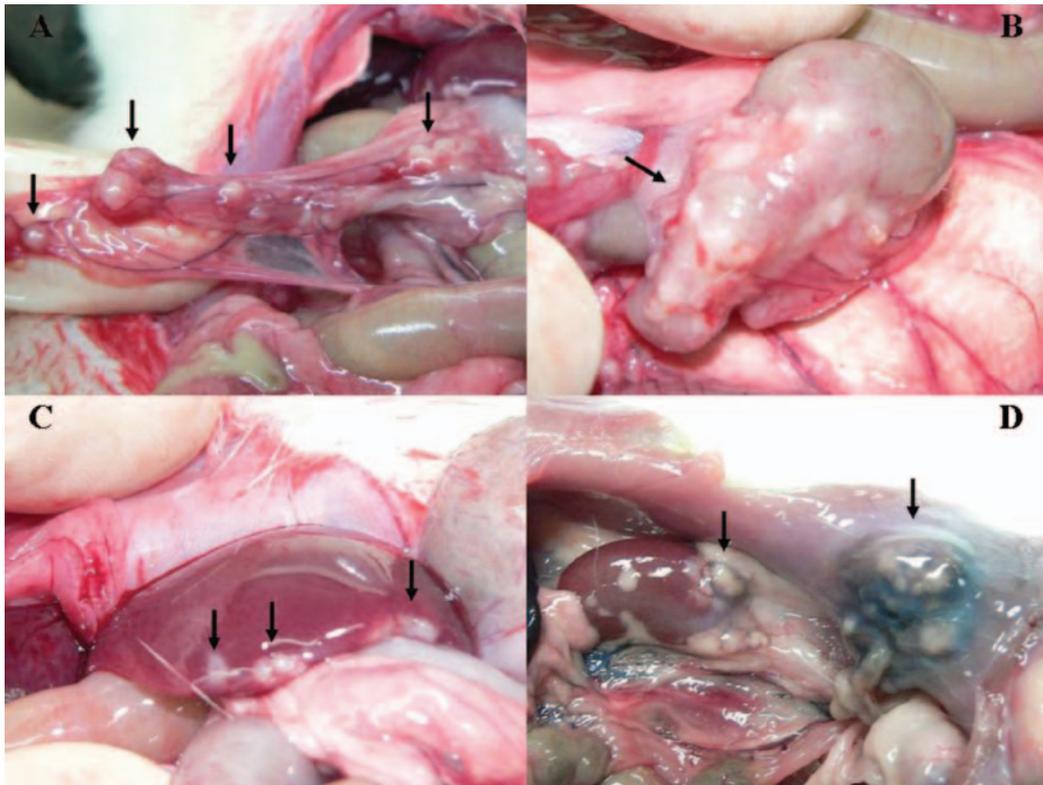


Figure 2. Tumour nodules in immunosuppressed rats injected with 50 million HT29 cells. Nodules on peritoneum (A) ,caecum(B), liver (C) and kidney (D).

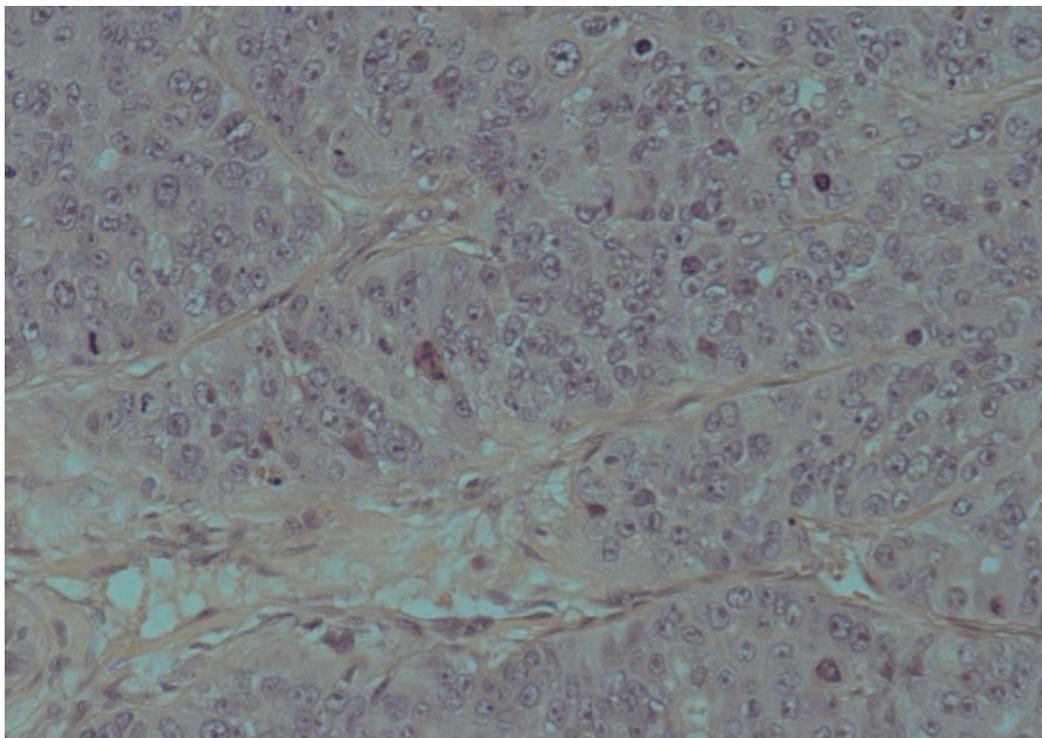


Figure 3. Tumour section of HT29 nodule. 20x H&E stain.

lymphoid irradiation to achieve a 100% take rate with mouse osteosarcoma, but results were much lower with human colon adenocarcinoma (13). In another study using cyclosporine at a daily dose of 55 mg/kg resulted in the animals' death in 5 days (8), however a dose of 35 mg/kg/day produced no signs of toxicity or premature death attributable to cyclosporine in the present study. Thus using only a single immunosuppressor, cyclosporine (35 mg/kg daily) a 100% take rate was achieved with the human colon adenocarcinoma cells in Sprague Dawley rats in this study which was in agreement to a previous study by Akla *et al.* (8) with human breast cancer cells. Goodman *et al.* (7), however, were unable to grow human melanoma cells in Lewis rats given cyclosporine at 15-50 mg/kg, but it is not clear whether that failure was due to the small inoculum size (1 million cell suspension) or to inadequate immunosuppression.

The encouraging results in this study may allow this model to be applied to larger animals. The tumours resulting from *i.p.* implantation of the human colon cancer cells were very invasive and in some rats penetrated the mucosa, blocking the lower intestine and prompting premature sacrifice. The extent of the disease progression was parallel to the inoculum size, with several rats injected with 50 and 100 million cells rapidly developing tumour-associated complications.

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