Antimetastatic effect of cimetidine on mice bearing a C3H mouse mammary adenocarcinoma: survival and lymphocyte function studies

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It has been reported that treatment with cimetidine, a histamine H2-receptor antagonist, increased survival and decreased the number of lung metastases in mice bearing the Lewis Lung carcinoma [29]. It was suggested that this effect was due to the ability of cimetidine to block histamine activation of suppressor lymphocytes and hence allow host defence mechanisms to inhibit tumour growth. In the present studies, C3H/He mice were implanted with a C3H mouse mammary adenocarcinoma on Day 0. This tumour metastasizes to the lungs in 30–50 days. Primary tumours were ablated with X-rays when they had grown to about 0.2 g and animals were given drinking water with or without cimetidine (10 mg ml−1) until the end of the experiment. Cimetidine reduced the number of mice dying from metastatic disease from 7/15 (controls) to 3/13. Cimetidine treatment also prolonged survival of mice that did succumb to metastatic disease by about 12 days. The response of spleen lymphocytes to the mitogens phytohaemagglutinin and lipopolysaccharide was assessed in vitro by uptake of 3H-thymidine 0, 16, 45 and 58 days after tumour implantation. Lymphocyte responsiveness was depressed by tumour burden. The influence of cimetidine treatment was equivocal being dependent upon time after tumour implantation and dose of mitogen. In this mouse-tumour system, the mechanism of the antimetastatic effect of cimetidine is different from that previously suggested [29].

Introduction

The presence of metastases at presentation, or their subsequent development, is one of the most common causes of failure in cancer treatment. There is no drug in current use that inhibits the process of metastasis that is not also a cytotoxic agent with attendant toxicity to normal tissues. An agent effective against metastases but which is also relatively non-toxic would be an important addition to the chemotherapists armamentarium.

Previous reports [13, 29] suggest that failure of the host to control the growth and metastatic development of immunogenic tumours might be due to, or at least associated with, an increase in non-specific suppressor cells active on cellular circuits that regulate effector-lymphocyte function. Osband et al. [29] characterized the suppressor cells as being histamine (H2) receptor positive lymphocytes which increased in numbers with, or as a consequence of, metastatic development, and whose activity was manifested when cultured with histamine. Prior treatment of
donor animals with cimetidine, a specific antagonist of H$_2$ receptors, blocked the activity of these lymphocytes, slowed metastatic development and increased survival of mice bearing the metastasizing Lewis lung carcinoma, 3LL (40 per cent of mice in the group treated with cimetidine at 10 mg ml$^{-1}$ were alive at a time when all controls had died). Cimetidine has been used to treat gastric and duodenal ulcers for over 10 years and it has few toxic side effects compared with conventional cancer chemotherapy agents. The possibility that a relatively non-toxic drug might be antimetastatic prompted the present study of the effect of cimetidine on mouse survival, using the C3H mouse mammary adenocarcinoma in syngeneic mice. An attempt was made to examine whether the inadequacy of the host's innate immunological 'surveillance system' could be accounted for by the accentuated appearance of a functionally active suppressor-cell population, similar to that arising with the 3LL tumour, and whether this suppression could be abrogated by treatment of the experimental animals with cimetidine given orally.

**Materials and methods**

The identity and transplantation details of the mouse-tumour model used in the present study has already been described [37]. Briefly, this C3H mouse mammary adenocarcinoma has been serially transplanted in syngeneic C3H/He mice since it arose spontaneously, more than 100 generations ago, in a female C3H/He mouse in the Gray Laboratory. The tumour is poorly differentiated. Viable areas consist mainly of large numbers of confluent tumour cells; occasional cords of tumour cells are seen around central blood vessels [19]. When grown subcutaneously in the back, the tumour metastasizes to the lungs in approximately half the implanted mice within 30–50 days. The number of mice developing metastases has increased gradually since the tumour arose [39]. Mice can be protected from developing metastases in this tumour model using the cytostatic antitumour agent razoxane (Penhaligon, unpublished) but the drug must be given from the day of implantation; treatment is less effective when primary tumour growth is more advanced [17]. The presence of circulating tumour-specific antibodies has not been assessed in this mouse-tumour model. For the present studies, 8–10-week-old, female, C3H/He mice were inoculated with $10^6$ tumour cells s.c. in the back in approximately 0·05 ml minimal essential medium (MEM, Gibco Europe Ltd) on day 0. When tumours reached approximately $6 \times 6 \times 4$ to $7 \times 7 \times 5$ mm$^3$ (diameter measured in three perpendicular directions)—usually day 14 to 18—mice were randomly allocated to control (tap water) and cimetidine treatment groups and the primary tumour ablated using 90 Gy X-rays. For tumour irradiation, mice were restrained in cylindrical, 3 mm thick lead jigs, without anaesthesia; the tumour protruding through a 3 mm-wide slit in the cylinder as described in detail in [37]. This ablation procedure was necessary since metastases in this tumour only become apparent by approximately 30–50 days post-implantation, by which time the tumour would have developed to an unacceptable size.

**Cimetidine**

Cimetidine was generously provided in powder form by Smith, Kline and French Laboratories Ltd, and dissolved (10 mg ml$^{-1}$) in tap water. Addition of a few drops of HCl was needed to dissolve the drug completely at this concentration. The pH remained approximately 6·5. Animals were given tap water or cimetidine ad libitum from the time of tumour irradiation until the end of the experiment.