P-glycoprotein (P-gp), a cell membrane protein, has been found in multidrug-resistant cancer cells. A total of 104 smears from patients with breast-cancer-associated pleural effusions and ovarian-cancer-related peritoneal effusions were studied for P-gp with the antibody C-219 and the avidin-biotin-immunoperoxidase method. Samples were taken before and 3 and 7 days after intracavitary bleomycin therapy and reaccumulation of effusion was assessed at 30 days. Smears that were P-gp-negative by the 7th day were associated with a good 30-day response to bleomycin in the majority of cases, while P-gp-positive smears were associated with a significant reaccumulation of fluid at 30 days. P-gp status is a valuable prognostic indicator of response to intracavitary bleomycin treatment in effusions from breast or ovarian cancer.

Key words P-Glycoprotein • Immunocytochemistry • Bleomycin • Drug resistance • Malignant effusions

Abbreviation P-gp P-glycoprotein

Introduction

Local management provides an effective treatment for control of malignant effusions. Intracavitary infusion of drugs may allow higher and more sustained drug levels at the site of disease than can be achieved by systemic administration (Ostrowski 1986; Hamed et al. 1989). On the other hand, tumour cell resistance to cytotoxic drugs is thought to be a major cause of failure of chemotherapy treatment of malignant tumours (Pastan and Gottesman 1987; Kartner and Ling 1989; West et al. 1994).

A membrane protein, P-glycoprotein (P-gp), is found in various multidrug-resistant cancer cells. This protein acts as a pump, effectively flushing chemotherapeutic agents out of the cell. The amount of this protein has been shown to be related to the degree of drug resistance (Juliano and Ling 1976; Fukua et al. 1987; Chatterjee et al. 1990; Weinstein et al. 1990; D’Incolci et al. 1991; Duensing et al. 1994). Recently several investigators have described the development of monoclonal antibodies with a high affinity for P-gp that proved useful in the detection of this protein (Ro et al. 1990; Rutledge et al. 1990; Athanassiadou et al. 1991; Verrelle et al. 1991; Roberts et al. 1995).

The aim of this study was to investigate the expression of P-gp in tumour cells from effusion smears after the intracavitary infusion of bleomycin for the control of malignant effusions and to correlate the findings with the effectiveness of the therapy.

Materials and methods

Effusion smears were studied from the pleural cavity of 64 patients with breast cancer and from the peritoneal cavity of 40 patients with ovarian adenocarcinoma before, and after 3 and 7 days of local infusion of bleomycin.

All patients studied had received initial surgical treatment of the primary tumour followed by six cycles of combination chemotherapy. Breast cancer patients received cyclophosphamide, methotrexate and 5-fluorouracil and ovarian cancer patients cisplatin, doxorubicin and cyclophosphamide.

Forty-three patients required second-line chemotherapy treatment consisting of either cyclophosphamide, 5-fluorouracil and doxorubicin or cisplatin and etoposide (for breast and ovarian cancer patients respectively).

All patients had received chemotherapy treatment for a minimum of 1 month before the incracavitary treatment, and at the time of bleomycin infusion systemic chemotherapy treatment was discontinued.
Patients with pleural effusions underwent thoracostomy tube drainage. After chest X-ray confirmation of complete fluid drainage, 60 mg bleomycin in 100 ml normal saline was instilled via the drainage tube. The tube was clamped and left for 24 h and then unclamped to allow continued drainage of the pleural cavity until such time as no further drainage occurred and not earlier than 7 days. The drain was then removed and the wound closed.

In the case of peritoneal effusions, abdominal paracentesis was performed with a Tenkoff catheter. All ascitic fluid was drained and 60 mg bleomycin in 100 ml normal saline was inserted into the peritoneal cavity, the catheter was removed at 7 days and the wound closed. Samples were taken from the drainage tube before, and after 3 and 7 days of bleomycin instillation.

For the detection of P-gp on smear cells, the monoclonal antibody C-219 (CIS Int) was used as the primary antibody in the avidin-biotin-immunoperoxidase method of Hsu et al. (1991). The smears were incubated with normal rabbit serum for 20 min, diluted 1:5 in phosphate-buffered saline (PBS), for blocking non-specific background. The primary antibody, C-219 (Cis Bio International, France), was applied at a previously determined optimal dilution for 30 min and incubated with biotinylated rabbit anti-(mouse immunoglobulins) (Dakopatts, Denmark) diluted 1:200 for 30 min at room temperature. Following thorough washing in PBS, the slides were incubated in streptavidin-biotin complex (Dakopatts, Denmark), used as recommended by the manufacturer. Diaminobenzidine (0.4 g/ml) with 0.03 % H2O2 in PBS was used to visualize the peroxidase enzyme (Sigma Diagnostics, UK). The resulting preparations were counterstained with Mayer’s haematoxylin, dried and mounted.

Smears of normal liver, stained in parallel, were used as positive controls and normal liver smears stained with the previously described technique, but using normal serum instead of the primary antibody, as negative controls.

Using light microscopy, only tumour cells exhibiting P-gp-specific membrane immunostaining, regardless of the number of cells stained, were considered as positive. These were scored on a four-point scale for intensity: 0 no staining, 1 weak but unequivocal staining, 2 definite staining of moderate intensity, 3 strong staining. Only tumour cells scoring 2 or more were considered P-gp-positive, regardless of the number of cells stained (Fig. 1). In all cases malignant cells were observed in the smears that were stained for P-gp at 0, 3 and 7 days. The cases in which no malignant cells were observed were excluded from the study.

The clinical response of each patient was evaluated as follows. (a) Complete response was defined as no further fluid collection as assessed by X-ray and negative cytology at or after 30 days; the fluid accumulation rate prior to the study ranged from 200 ml/week to 300 ml/week. (b) Partial response was minimal fluid accumulation with positive cytology. (c) Minimal response meant more than 50 % reduction in the rate of fluid accumulation with positive cytology. (d) No response meant little or no reduction in the rate of fluid accumulation and positive cytology.

Statistical analysis of the results obtained was performed using $\chi^2$ analysis and Yates' correction for small numbers.

Fig. 1A, B Immunocytochemical expression of P-glycoprotein. A Ovarian adenocarcinoma cells stained with C-219 showing various degrees of membrane staining. B Breast adenocarcinoma cells reacted with C-219. Tumour cells exhibit pale to dark membrane staining.