Sequential immunochemotherapy and edrecolomab in the adjuvant therapy of breast cancer: Reduction of 17-1A-positive disseminated tumour cells

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Received 25 April 2001; revised 7 January 2002; accepted 11 February 2002

Background: The aim of our study was to evaluate the efficacy of the monoclonal antibody edrecolomab after chemo- and radiotherapy in the elimination of disseminated tumour cells in bone marrow in the adjuvant therapy of breast cancer.

Patients and methods: The bone marrow of 25 patients with breast cancer was tested for the presence of disseminated tumour cells using the pancytoceratine antibody and the alkaline phosphatase-antialkaline-phosphatase (APAAP) technique. To characterize tumour cells simultaneously, immunofluorescent double labelling of pancytoceratine and epithelial cell adhesion molecule (antibody 17-1A) was performed on tumour cells after magneto bead enrichment. Patients positive for the 17-1A antigen in bone marrow after chemotherapy were treated with edrecolomab (500 mg Panorex® initially, then 100 mg/month over 4 months) and investigated for the presence of micrometastases 6 weeks after the last treatment.

Results: Of the 17 patients showing bone marrow micrometastases (BM-MM), 14 tested 17-1A positive before adjuvant chemotherapy. After chemotherapy, nine patients remained positive for the 17-1A antigen and were treated with edrecolomab. The final investigation after immunotherapy showed a complete elimination of the 17-1A-positive BM-MM in seven patients and a significant reduction of these cells in two patients.

Conclusions: Sequential treatment of breast cancer with edrecolomab after adjuvant chemotherapy can reduce disseminated tumour cells in the bone marrow and eliminate 17-1A-positive micrometastases.

Key words: 17-1A antigen, bone marrow, breast cancer, disseminated tumour cells, immunotherapy

Introduction

The adjuvant chemotherapy or hormonal blockade in early-stage breast cancer leads to a significant increase of overall survival and disease-free survival. Positive lymph nodes and disseminated tumour cells are described as prognostic factors limiting the positive chemotherapeutic effect. Although mortality has been lowered in the last few years, recurrences have been seen in 35% of patients within 10 years, and still up to 40% of patients will ultimately die from metastases [1].

There is emerging evidence that the presence of disseminated tumour cells in bone marrow is a prognostic factor in cancer, particularly in breast cancer [2–6]. Epithelial tumour cells are able to be disseminated to secondary organs at an early stage of primary tumour development. The data that are currently available suggest that bone marrow micrometastases (BM-MM) represent a select population of dormant cells that can lead to early relapses of the disease [6].

The efficacy of chemotherapy on these tumour cells is limited because of their predominantly dormant non-proliferating status [7–9]; therefore, adjuvant treatment against these tumour cells is of great interest. Treatment with the monoclonal antibody edrecolomab has been reported to kill quiescent tumour cells, despite their resistance to chemotherapy [10]. Nevertheless, the potential benefit of edrecolomab is controversial. While Riethmüller et al. demonstrated its efficacy in a 7-year follow-up, with a 32% reduction in the overall mortality in colon cancer patients [11, 12], Punt et al. [13] demonstrated that the addition of edrecolomab to 5-FU/LV in the adjuvant treatment of stage III colon cancer did not improve overall survival. Braun investigated the efficacy of edrecolomab on disseminated tumour cells in 10 patients with advanced breast cancer in the metastatic state, and showed a complete elimination of epithelial cell adhesion molecule (EpCAM)-positive cells in four patients [14, 15].
The aim of our study was to evaluate the efficacy of the monoclonal antibody edrecolomab in the elimination of disseminated tumour cells in bone marrow in the adjuvant therapy of breast cancer.

Patients and methods

From June 1997 to December 1999, 25 patients with a diagnosis of breast cancer were consecutively assigned to our study after written informed consent was received. Criteria for inclusion was the adjuvant situation (TNM stage N0–2 M0 R0). First, the prevalence of BM-MM was monitored using BM samples obtained by BM aspiration (6 ml) from each spin iliaca posterior under local anaesthesia, before and after adjuvant chemotherapy [ATC (doxorubicin, paclitaxel and cyclophosphamide), ADM (doxorubicin) + CMF (cyclophosphamide, methotrexate and 5-fluorouracil), CMF, CMF + Taxol, EC (epirubicin and cyclophosphamide, methotrexate and 5-fluorouracil) CMF, CMF, + Taxol, EC (epirubicin and cyclophosphamide), EC + Taxol]. Bone marrow biopsy was done within 2 weeks of surgery and within 4 weeks of chemotherapy. Chemotherapy was completed on average 24 weeks after the first marrow sample extraction (median 24 weeks, range 20–28 weeks). The tumour tissue was handled only when paraffin embedded.

BM-MM were detected by pancytoceratine antibody (A45-B/B3) and visualized by the alkaline phosphatase–anti-alkaline-phosphatase technique (APAAP) as described by Cordell et al. [16] and Braun et al. [14]. To characterize tumour cells, simultaneous immunofluorescent double labeling of pancytokeratine and EpCAM (antibody 17-1A) was performed on tumour cells after magneto bead enrichment as described by Naume et al. [17] and Braun et al. [15]. After permeabilization with 0.1% Triton X-100 and fixation with 1.0% paraformaldehyde, the immunocytochemical double-labeling technique was performed by a simultaneous incubation with alkaline phosphatase-conjugated anti CK A45-B/B3 Fab fragments and the anti EpCAM immunoglobulin edrecolomab. After co-incubation of the antibody conjugates, specific goat antimouse immunoglobulins conjugated with colloidal gold particles were added to specifically label murine edrecolomab [15]. After washing steps and fixation, silver development was carried out and terminated under the microscope as soon as silver precipitates became visible.

In cases of cytokeratine-positive BM-MM with 17-1A antigen expression after the adjuvant chemotherapy, the efficacy of an additional therapy with the monoclonal antibody 17-1A edrecolomab was investigated.

The initial dose of edrecolomab (Panorex®) was 500 mg, followed by 100 mg/month over 5 months.

Main target parameters of the study were the evidence of disseminated tumour cells in newly diagnosed breast cancer patients, and the prevalence of BM-MM with 17-1A antigen expression before and after adjuvant chemotherapy and additional immunotherapy. In addition, the tolerance of patients to immunotherapy and the clinical follow-up were investigated.

Results

In a total of 25 patients, 17 women were found to have BM-MM after surgery. Fourteen out of 17 patients (TNM stage: T1 eight patients and T2 six; N0 four patients and N1 10; G1 one patient, G2 11 and G3 two) were positive for the 17-1A antigen prior to adjuvant chemotherapy (Table 1). Nine of these 14 patients were hormone receptor positive, two were negative and three were unknown. The patients underwent chemotherapy following the ATC protocol in six cases, ADM + CMF in four cases, CMF in six cases, CMF + Taxol in two cases, EC in five cases, and EC + Taxol in two cases. After the adjuvant chemotherapy, six out of 14 patients (43%) were positive for BM-MM according to Epimed testing. Nine out of 14 patients (64%) were proven positive after tumour cell enrichment for the 17-1A surface antigen (Table 2). One of the 14 patients (7%) was positive after tumour cell enrichment, but negative for the 17-1A surface antigen. This means that after chemotherapy, in four out of 14 patients (29%), disseminated tumour cells could be eliminated completely as proven by the Epimed test as well as by magneto bead enrichment.

Table 1. Presence of BM-MM before adjuvant chemotherapy in the 14 patients of 17 demonstrating 17-1A-positive BM-MM

<table>
<thead>
<tr>
<th>APAAP</th>
<th>Tumour cell enrichment</th>
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<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Negative Positive 17-1A-positive 17-1A-negative</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
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<tr>
<td>14</td>
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n = 14 (T1 n = 8, T2 n = 6; N0 n = 4, N1 n = 10; G1 n = 1, G2 n = 11, G3 n = 2; M0).

Table 2. Presence of BM-MM after adjuvant chemotherapy in the 14 patients of 17 demonstrating 17-1A-positive BM-MM

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<tr>
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</tr>
<tr>
<td></td>
<td>Negative Positive 17-1A-positive 17-1A-negative</td>
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<tr>
<td>6</td>
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<td>4</td>
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n = 14 (T1 n = 8, T2 n = 6; N0 n = 4, N1 n = 10; G1 n = 1, G2 n = 11, G3 n = 2; M0).
After chemotherapy and radiotherapy, all nine women presenting 17-1A-positive BM-MM (Table 3) were treated with edrecolomab. Six of these nine patients were hormone receptor positive, one was negative and two were unknown; three of these nine patients were premenopausal, four postmenopausal and two menopausal.

In these nine patients (TNM stage: T1 four patients and T2 five; N0 five patients and N1 four; G2 eight patients and G3 one), the presence of BM-MM was monitored 4 weeks after antibody treatment (Table 4). Five of the nine women (56%) were negative for BM-MM according to Epimed testing before tumour cell enrichment. Two of six patients positive by Epimed testing (33%) showed an elimination of BM-MM after the immunotherapy. Complete elimination of the 17-1A-positive cells was found after tumour cell enrichment in seven out of nine women (78%). In two out of nine patients (22%), a significant reduction of ∼75% of the 17-1A-positive BM-MM was evident. Nevertheless, all nine patients remained positive for 17-1A-negative disseminated tumour cells after tumour cell enrichment.

Tolerance of the patients to immunotherapy was excellent in all patients. There was no fever, no allergic reaction, no neutropenia or gastrointestinal side effects. All patients were still alive at a mean follow-up of 33.4 months (median 30 months, range 20–46 months) and no evidence of relapse was found. In one patient we diagnosed small cell lung cancer as a second tumour entity during the follow-up.

### Discussion

Prospective clinical studies of the detection of BM-MM have identified patient subgroups with a poor clinical prognosis with regard to early metastatic manifestation and reduced overall survival in various epithelial tumour entities including breast cancer [2, 3, 6, 18]. The elimination of these mainly non-proliferating ‘dormant’ cells should therefore be of great importance, since chemotherapies have largely been proven to be ineffective [7, 8, 19, 20].

New therapeutic strategies to prevent metastatic relapse in patients with operable primary carcinomas, such as immunotherapy with the monoclonal antibody edrecolomab, are still controversial [11–13].

Edrecolomab is a mouse-derived monoclonal IgG 2a antibody [10, 21]. It recognizes the human tumour-associated antigen Co 17-1A, which is expressed on the surface of a wide variety of tumour cells, and appears to be related to the presence of well differentiated tumours [10].

The immunotherapeutic agent is thought to destroy tumour cells by activating an array of endogenous cytotoxic mechanisms, including antibody-dependent cell-mediated cytotoxicity and possibly antibody-dependent complement-mediated cytotoxicity. Edrecolomab may induce antitumour activity indirectly by inducing a host anti-idiotypic response.

In clinical practice, edrecolomab was first used by Riethmüller et al. [11] for the adjuvant treatment of colon cancer. In their study, the intention-to-treat analysis showed a significant effect on overall and disease-free survival, with a decreased recurrence rate of 25% and a 32% reduction in overall mortality in colon cancer patients. The presence of the 17-1A surface adhesion molecule on disseminated tumour cells, identified by immunocytochemical methods, has been demonstrated in 50% of breast cancer patients and 60% of disseminated tumour cells.

Braun et al. [14] showed how a single edrecolomab application (1 × 500 mg) in patients with metastatic breast cancer

### Table 3. Presence of BM-MM in nine patients before additional immunotherapy with edrecolomab (Panorex®)

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<tr>
<td></td>
<td>Positive</td>
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<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>17-1A-positive</td>
<td>6</td>
</tr>
<tr>
<td>17-1A-negative</td>
<td>9</td>
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</tbody>
</table>

n = 9 (T1 n = 4, T2 n = 5; N0 n = 5, N1 n = 4; G2 n = 8, G3 n = 1; M0).

### Table 4. Presence of BM-MM in nine patients after additional immunotherapy with edrecolomab (Panorex®)

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<thead>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>17-1A-positive</td>
<td>4</td>
</tr>
<tr>
<td>17-1A-negative</td>
<td>2</td>
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*Significant reduction in number of cells (75%).

n = 9 (T1 n = 4, T2 n = 5; N0 n = 5, N1 n = 4; G2 n = 8, G3 n = 1; M0).
could result in 17-1A-positive tumour cells being significantly reduced in four of the eight patients tested and eliminated completely in the remaining four. We have confirmed these results in this study of nine patients with 17-1A-positive BM-MM in the adjuvant breast cancer setting, using the recommended multiple application of edrecolomab (1 × 500 mg plus 4 × 100 mg). No correlation could be found between tumour grading, tumour size or the presence of metastatic enlarged lymph nodes and the presence of disseminated tumour cells before edrecolomab therapy. Bearing in mind the small number of patients in our study, our results demonstrate that use of the recommended multiple dose regimen of edrecolomab could achieve a complete elimination of 17-1A-positive BM-MM in 78% of patients and a significant reduction in 22%. The additional benefit of 28% compared with Braun’s investigation (which showed only 50% complete elimination) may be due to the multiple dose application of edrecolomab. The immunotherapy was very well tolerated and had no side effects.

Despite the results discussed above, none of the nine patients showed a complete reduction in the number of disseminated tumour cells after tumour cell enrichment. Nevertheless, the prognostic value of the evidence of the cells by means of this method has not yet been fully determined; for example, Mansi et al. described a spontaneous loss of BM-MM over time [22]. We showed using APAAP immunostaining that five of the nine patients (56%) were completely negative for BM-MM after edrecolomab therapy. If this testing alone were considered to have a prognostic value, the complete elimination of 56% of BM-MM would demonstrate that cure of breast cancer by means of additional immunotherapy might be possible. On the other hand tumour cell enrichment, now valuable for improving detection of disseminated tumour cells, showed only a selective elimination of 17-1A-positive BM-MM, an argument against the statement of Mansi et al. [22]. The selective disappearance of 17-1A-positive tumour cells is unlikely to have occurred spontaneously in patients with BM-MM. Taking into consideration that fact that two of our nine patients showed a significant reduction in the number of 17-1A-positive tumour cells, our results also revealed evidence of other 17-1A-negative tumour cells in bone marrow after immunotherapy using an immunomagnetic technique. The efficacy of edrecolomab is evidently due to its selective elimination of 17-1A-positive cells. This may be explained by Braun’s theory that the tumour cell antigen heterogeneity of disseminated tumour cells limits the efficacy of monovalent immunotherapeutic strategies directed against only one particular antigen. Therefore, new immunotherapeutic strategies should be developed to detect and eliminate these cells [7].

Because of the small number of patients we cannot definitely exclude that some of them could have been negative purely by chance or methodological variability. On the other hand it seems more unlikely that the elimination of 17-1A-positive cells after antibody application occurred purely by chance.

The follow-up to our data will demonstrate the significance of both tumour cell enrichment and APAAP immunostaining with regard to the prognostic value.

One limitation of our study is that we cannot definitely exclude a biased endocrine effect of adjuvant chemotherapy. An additional endocrine effect of chemotherapy-induced cessation of ovarian function may in part explain the delayed disappearance of 17-1A-positive cells, particularly since six out of nine patients were hormone receptor positive.

In conclusion, sequential treatment of breast cancer with edrecolomab after adjuvant chemotherapy can reduce the number of tumour cells in bone marrow and eliminate 17-1A-positive cells in breast cancer. This therapy is very well tolerated, which is highly important in the adjuvant situation. Further investigations are ongoing, correlating these data with disease-free and overall survival of the patients in this study.

References


