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ORIGINAL ARTICLE

The predictive role of Bcl-2 expression in operable locally advanced or metastatic gastric carcinoma

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Summary

Purpose: *Gastric carcinoma is an aggressive disease with different epidemiologic and clinical profiles. Combination chemotherapy containing docetaxel, cisplatin and 5-fluorouracil/5-FU (DCF) is a frequently used regimen in metastatic gastric cancer. We studied the role of B-cell lymphoma 2 (Bcl-2) expression in predicting the response to DCF combination chemotherapy in metastatic gastric carcinoma.*

Methods: *This study included patients with pathologically confirmed locally advanced, surgically inoperable gastric carcinoma, or with metastatic disease. For immunohistochemical staining of Bcl-2 oncoprotein, lyophilized mouse monoclonal antibody (clone100/D5, 1:50, Thermo Scientific, Fremont, ABD) was used. Bcl-2 expression was evaluated with respect to the nuclear and cytoplasmic staining of the cells. Staining > 10% was accepted as positive and ≤ 10% as negative.*

Results: *Bcl-2 expression was positive in 5 (23.8%) patients and negative in 16 (76.2%), while partial response was achieved in 12 (57%) patients. No complete response was seen in any patient. The effect of positive Bcl-2 expression on survival was statistically significant by log-rank test (p=0.035).*

Conclusion: *The patient group that expressed Bcl-2 survived longer confirming that Bcl-2 expression is a good prognostic factor in advanced-stage patients. We believe that Bcl-2 expression has an additional contribution in predicting response to this chemotherapy combination.*

Key words: Bcl-2, chemotherapy, gastric cancer, prediction of response

Introduction

Gastric carcinoma is an aggressive disease with different epidemiologic and clinical profiles in different parts of the world. It accounts for approximately 9.9% of all new cancer cases [1] and ranks second as cause of death from cancer [2]. In Turkey, it is the second most frequently seen form of cancer in men, and the third most frequently seen in women [3].

Early-stage gastric carcinoma has a relatively more mild course when compared to advanced stages. In early-stage gastric carcinoma, surgery and adjuvant chemoradiotherapy are applicable [4]. Metastatic gastric carcinoma shows rapid progression. While survival is about 3-5 months in the absence of chemotherapy, with combination chemotherapy it may be extended to about 8-12 months [5]. DCF combination chemotherapy is a frequently used regimen in the metastatic setting. This combination, however, can produce serious side effects. Grade 3-4 neutropenia has been reported in 75-81% of the patients [6]. During treatment, mortality due to all causes is about 7-9% [6]. Prediction of response to this combination chemotherapy would possibly protect some patients from treatment complications and hinder the loss of some others from causes associated with treatment complications.

The loss of balance between apoptosis and proliferation also plays a role in gastric carcinoma genesis. Bcl-2 is a member of a protein family that plays an important role in the apoptotic mechanisms. Changes in Bcl-2 expression have been shown in 12-35% of patients with gastric carcinoma. In patients with Bcl-2 overexpression, a less aggressive biological behavior has been reported, which has been attributed to the suppression of cell proliferation by Bcl-2 [7-10].

Prognosis may be established on the basis of certain

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clinical and laboratory parameters obtained during diagnosis. Determining the mechanisms that play a part in oncogenesis for each patient may suggest the treatment to be chosen. Laboratory findings may be used for deciding on the treatment intensity to be used for each patient. In this article we studied the role of Bcl-2 expression in predicting the response to DCF combination chemotherapy given by continuous infusion over 24 h in patients with locally advanced or metastatic gastric carcinoma.

Methods

Patients

The study encompassed patients with locally advanced gastric carcinoma, surgically inoperable, or metastatic confirmed pathologically in the Medical Oncology Clinic of Antalya Training and Research Hospital between 2008-2010. Patients with ECOG performance status (PS) 0-2, objectively measurable disease, sufficient bone marrow reserve and normal hepatic and renal function were recruited. Patients with an ECOG PS > 3 and those who were under treatment in our clinic following a period of treatment started in other institutions were excluded from study. Patient files were reviewed retrospectively and data were obtained in relation to stage of disease and treatment given.

Chemotherapy

A chemotherapy protocol consisting of docetaxel 75 mg/m^2, day 1, cisplatin 75 mg/m^2 day 1 and 5-FU 1000 mg/m^2 days 1-5, given by continuous infusion over 24 h, was administered for a minimum of one cycle. Cycles were repeated every 21 days.

Immunohistochemical studies

All specimens were obtained from tissues fixed by formalin and embedded in paraffin. Paraffin blocks were cut into 4 μm sections. After deparaffinization of the sections gradual rehydration was carried out with ethanol and the procedure for Bcl-2 staining was performed. In staining the specimens for Bcl-2 oncoprotein, lyophilized mouse monoclonal antibody (clone100/D5, 1:50, Thermo Scientific, Fremont, ABD) was used. Following staining, the specimens were studied with Nikon Eclipse 80i microscope.

Immunohistochemical scoring

Expression rates for the Bcl-2 positive tumor cells in the specimens were evaluated by two pathologists who had no knowledge of the patients' clinical features. Bcl-2 expressions were evaluated according to nuclear and cytoplasmic staining in the cells (Figures 1,2).

Statistical analysis

Statistical analysis was carried out using the SPSS software program. Immunohistochemically positive and negative results were individually studied with respect to survival, using the Kaplan-Meier survival analysis. Statistical differences were validated with the log-rank test. The level of significance was set at <0.05.

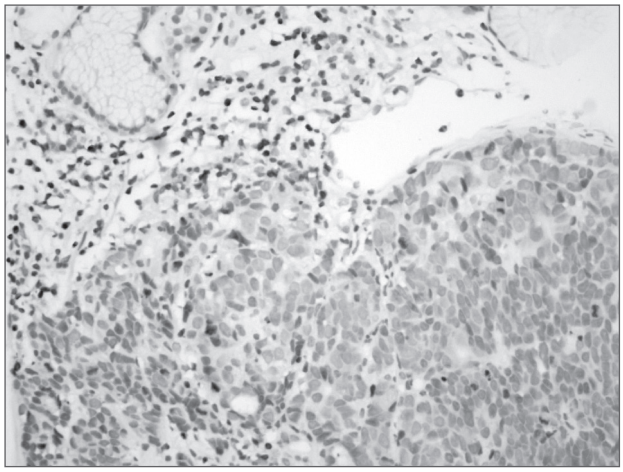

Figure 1. Bcl-2 negative gastric cancer.

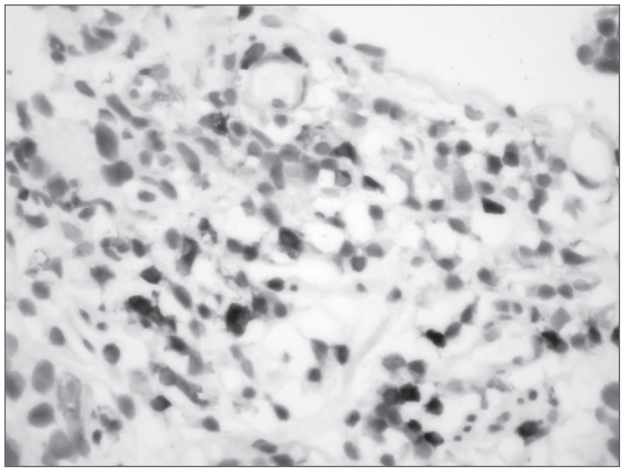

Figure 2. Bcl-2 positive gastric cancer.

Results

A total of 21 patients entered the study, of whom 17 (81%) were male and 4 (19%) female. The median patient age was 64 years (range 34-77; Table 1). Ten patients (47.6%) were considered surgically inoperable. Three patients (14.3%) underwent only laparoscopic biopsy. One patient underwent partial gastrectomy, 2 patients were subjected to total gastrectomy, and one patient was subjected to subtotal resection. According to either preoperative or postoperative evaluation results, all of the patients were classified as having stage IV disease. Thirteen (52.4%) patients were diagnosed with liver metastasis, 7 (33.3%) were found with peritoneal carcinomatosis, one patient had peritoneal carcinomatosis with liver metastasis, and 2 patients had non regional lymph node metastasis.

The patients received a mean of 4 courses of chemotherapy (range 1-6). Hematological toxicity included grade 3-4 neutropenia in 8.3% of the patients, grade 3-4

Table 1. Patient characteristics

Characteristics	*Mean (±SD)*	*Median*
Age (years)	60.9±11.6	64
Height (cm)	158.2±5.3	158.5
BUN (mg/dl)	20.6±9.8	18
CRE (mg/dl)	1.02±0.09	1
AST (U/L)	20.2±7.6	18
ALT (U/L)	24.7±8.2	19
LDH (U/L)	215.3±78	196
ALP (U/L)	93.2±32.9	90
WBC (10^3/mm^3)	6724±2804	6740
PLT (10^3/mm^3)	299,587±137,947	264,000
Hb (g/dl)	11.4 ± 1.3	11.4

BUN: blood urea nitrogen, CRE: creatinine, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, WBC: white blood cells, PLT: platelets, Hb: hemoglobin, SD: standard deviation

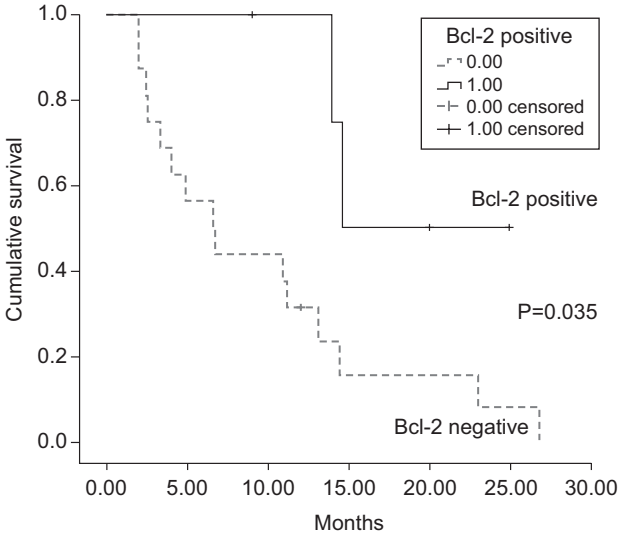

Figure 3. Overall survival in relation to Bcl-2 expression.

anemia in 7%, and grade 3-4 thrombocytopenia in 6%. Non-hematological grade 3-4 toxicity occurred in 5% of the patients as nausea and vomiting, and 5.2% as diarrhea. Two patients developed febrile neutropenia; they were hospitalized and given antibiotic treatment. There were no chemotherapy-associated deaths.

While partial response was achieved in 12 (57%) patients, there was no patient with complete response. Seven patients showed progressive disease. Two patients died before response evaluation. The relationship between Bcl-2 expression and response to treatment was statistically significant (p=0.03). While partial response was found in all of the patients with Bcl-2 expression, partial response was also achieved in 7 patients in the group without such expression.

Bcl-2 expression was positive in 5 patients (23.8%), and negative in 16 (76.2%). The median follow-up period was 10.8 months (range 1.9-26.8). Without taking Bcl-2 expression into account, the median overall survival was 11.2 months (range 8.1-15.9). The median survival was 14.6 for the patients with Bcl-2 expression, while it was 6.6 months for those without Bcl-2 expression. The effect of the presence of Bcl-2 expression on survival was statistically significant by log-rank test (p=0.035; Figure 3).

Discussion

We examined the role of Bcl-2 expression in predicting the response to a combination of docetaxel, cisplatin and 5-FU in patients with inoperable, locally advanced and metastatic gastric carcinoma. Bcl-2 expression was found in 23.8% of the patients. The median overall survival of the patients with Bcl-2 expression was significantly longer than those without (14.6 vs. 6.6 months; p=0.035).

Bcl-2 gene was first recognized through its involvement in t(14;18) translocation in patients with B-cell follicular lymphoma. Its expression, however, is not dependent only on this location. Bcl-2, by inhibiting apoptosis, extends the cells' lifespan and causes more encounters with mutagenic factors [11]. Saesuge et al. have shown that Bcl-2 expression is low in regions where Ki-67 index is high in gastric biopsies [12]. Perrone et al. found that vascular endothelial growth factor expression in patients with colon cancer is associated with Bcl-2 expression [13]. Although Bcl-2 plays a part in carcinogenesis, it is associated with a less aggressive course in many forms of cancer. It has been demonstrated that increased Bcl-2 expression is a good prognostic factor in non-small cell lung cancer, breast cancer, ovarian cancer, soft tissue sarcomas and colon cancer [14-17]. However, in some forms of cancer it has been shown to be a factor for poor prognosis. It has been reported that increased Bcl-2 expression in patients with prostate cancer is associated with resistance to hormonal treatment and recurrence, and in breast cancer patients it is associated with poor prognosis [18,19].

Bcl-2 protein family is being evaluated also for targeting treatments [20]. Kim et al. have shown that antisense Bcl-2 *in vivo* and *in vitro* increases sensitivity to paclitaxel, cisplatin and doxorubicin [21].

Bcl-2 expression shows differences between the early and advanced stages of gastric carcinoma patients. While Lee et al. found the rate of Bcl-2 expression as 12.7% among patients with early and advanced-stage gastric carcinoma, this rate was recorded as 32.6% among advanced-stage patients in another study [9]. In our study, the rate of Bcl-2 expression was 23.8%. We consider this as the result of the fact that our study

group consisted of patients with inoperable locally advanced or with metastatic disease. In both of the previously mentioned studies 5-year survival rate was longer in patients with increased Bcl-2 expression, leading to the conclusion that Bcl-2 expression is a good prognostic factor for gastric carcinoma [11].

Changes in Bcl-2 expression after chemotherapy have also been investigated. Serial biopsies have shown that Bcl-2 expression decreases in breast cancer patients treated with docetaxel and doxorubicin [22]. In another study, a correlation was shown between Bcl-2 overexpression and survival in non-small cell lung cancer patients who were treated with synchronous chemoradiotherapy containing cisplatin [23]. These studies suggest that Bcl-2 expression can predict response to chemotherapy, as it was also shown in our study.

We found that the patient group that expressed Bcl-2 responded better to the chemotherapy administered. It is known that Bcl-2 expression is a good prognostic factor in advanced-stage patients with gastric cancer. We believe that Bcl-2 expression has an additional contribution in predicting the response to this chemotherapy combination which has a series of rather severe toxic effects.

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