In vitro chemosensitivity to gemcitabine, oxaliplatin and zoledronic acid predicts treatment response in metastatic gastric cancer

Jörg Trojan[a], Soo-Zin Kim[b], Knut Engels[c], Susanne Kriener[c], Paris S. Mitrou[b] and Kai U. Chow[b]

Individual response of disseminated cancer to chemotherapy is unpredictable. *In vitro* chemotherapy-induced apoptosis can be measured and might be a method to evaluate *in vivo* activity of tested drugs. In this report, tumor cells of a patient with signet cell carcinoma of the stomach and diffuse bone marrow infiltration were cultured and tested for *in vitro* chemosensitivity. The drugs gemcitabine, oxaliplatin and zoledronic acid were found to induce *in vitro* tumor cell apoptosis synergistically, and subsequently were used as combination chemotherapy regimen. An initially existing disseminated intravascular coagulopathy quickly resolved and after 6 months of treatment on ongoing complete response was induced, thus confirming the results of *in vitro* chemosensitivity testing. *Anti-Cancer Drugs* 16:87–91 © 2005 Lippincott Williams & Wilkins.

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[a]Division of Gastroenterology, [b]Division of Hematology and Oncology and [c]Senckenberg Center of Pathology, Department of Internal Medicine, Johann Wolfgang Goethe University Medical Center, Frankfurt, Germany.

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Correspondence to J. Trojan, Division of Gastroenterology, Department of Internal Medicine, Johann Wolfgang Goethe University Medical Center, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany.
Tel: +49 69 6301 7860; fax: +49 69 6301 83776;
e-mail: trojan@em.uni-frankfurt.de

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Introduction

Adenocarcinoma of the stomach is one of the most frequent causes of cancer death worldwide. In patients with metastatic gastric cancer, palliative chemotherapy based on a 5-fluorouracil/platinum combination is a widely accepted regime, with response rates of 25–40% and a median overall survival of 7–9 months [1]. However, it is not possible to predict which patients benefit from palliative chemotherapy. Diffuse bone marrow metastasis leading to hematological disorders, such as disseminated intravascular coagulopathy (DIC) and microangiopathic hemolytic anemia, occurs in a subset of patients with advanced gastric cancer, and is indicative of a very poor prognosis with a median survival of only 2 months [2].

Case report

Clinical and laboratory findings

A 67-year-old male patient presented with fatigue and loss of appetite. The initial blood count revealed a normochromic, normocytic anemia and a thrombocytopenia. Further laboratory work-up showed decreased haptoglobin, elevated levels of indirect bilirubin, lactate dehydrogenase, elevated reticulocyte count and prolonged prothrombin time, confirming a hemolytic anemia with signs of DIC. For further work-up the patient was admitted to hospital and a bone marrow biopsy was performed, showing a diffuse infiltration of signet cells, involving 60% of the biopsy specimen (Fig. 1a). The carcinoma infiltration of the bone marrow was visualized by immunohistochemistry using an anti-pan-cytokeratin antibody (clone MNF116; Dako, Carpinteria, CA) as primary antibody and a LSAB system with Fast-red as chromogen (Super Sensitive MultiLink; Biogenex, San Ramon, CA).

Diagnostic imaging

Subsequently, an upper-gastrointestinal endoscopy confirmed a circumferentially growing signet cell carcinoma of the antrum (Fig. 1b). Because of anemia and thrombocytopenia due to progressive DIC, the patient required repeated blood transfusions the following days. Local staging by endoscopic ultrasound and computed tomography (CT) scanning revealed a cT3 cN2 tumor of the antrum infiltrating the pyloric channel. A [^{99m}Tc]DPD bone scan showed a diffuse enrichment in the skull, proximal humerus, the total spine and the whole pelvic region including both proximal femurs. A CT scan further demonstrated osteolytic metastases of the pelvis. Thus, the tumor was considered a cT3 cN2 pM1 (bone marrow) signet cell carcinoma of the antrum with DIC.

In vitro chemosensitivity testing

After signed informed consent, a bone marrow aspirate was taken according to an open phase II protocol, currently investigating the impact of *in vitro* chemosensitivity testing prior to scheduled chemotherapy in patients with leukemic non-Hodgkin's lymphoma

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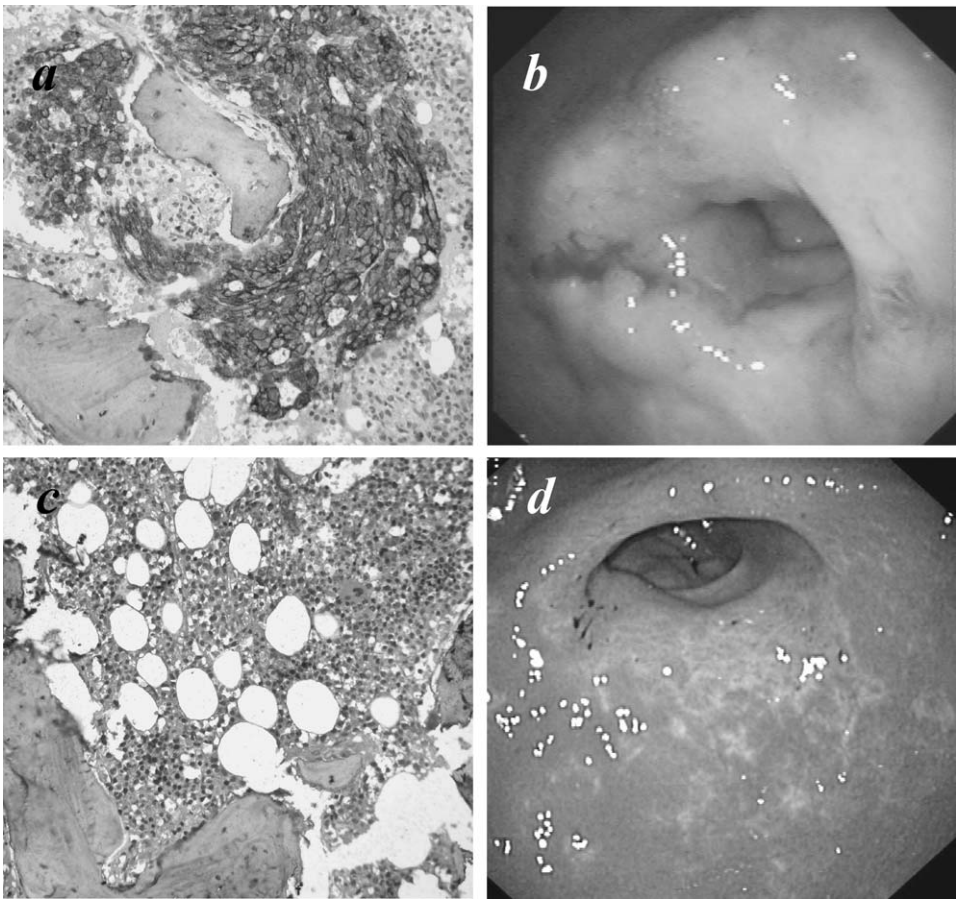
Fig. 1



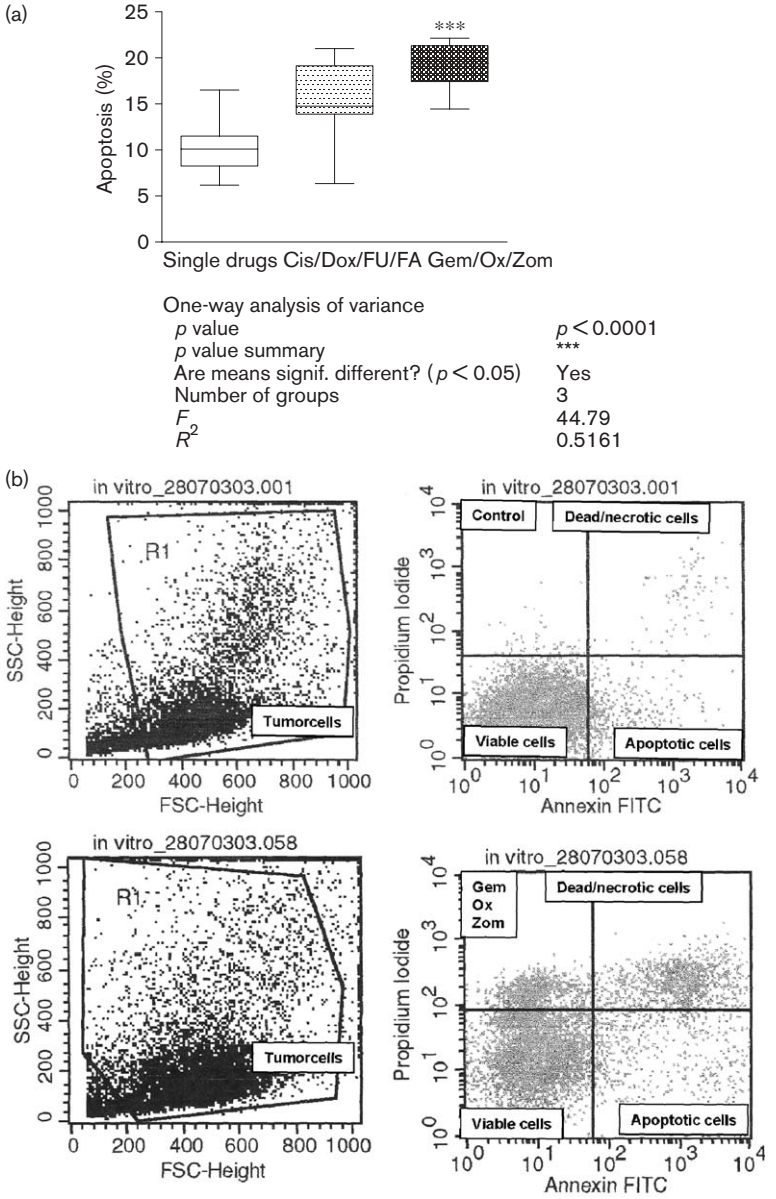
Findings in a patient with advanced signet cell carcinoma of the stomach and disseminated intravascular coagulopathy. (a) Bone marrow biopsy, showing a diffuse infiltration of signet cells, involving approximately 60% of the specimen (pan-cytokeratin staining, ×200). (b) Upper gastrointestinal endoscopy, showing a vulnerable, circumferentially growing signet cell carcinoma of the antrum. (c) Bone marrow biopsy after 6 months of combination chemotherapy with gemcitabine, oxaliplatin and zoledronic acid, which induced complete resolution of the disseminated intravascular coagulopathy. No more signet cells were detectable (pan-cytokeratin staining, ×200). (d) Upper-gastrointestinal endoscopy after 6 months showing no residual tumor, but scar tissue, which was confirmed by histology.

at the University Medical Center of Frankfurt/Germany. Tumor cells were selected by a Ficoll gradient, cultured, and incubated with cytotoxic agents in various concentrations and combinations for 24 h. The percentage of apoptotic cells was assessed by flow cytometry using Annexin-V as described previously [3]. The drugs oxaliplatin (dose range 1–15 μg/ml), cisplatin (dose range –15 μg/ml), gemcitabine (dose range 5–10 μg/ml), doxorubicin (dose range 0.5 μg/ml), 5-fluorouracil (5-FU) (dose range 2.5–10 μg/ml), folinic acid (dose range 20–100 μg/ml) and the bisphosphonate zoledronic acid (dose range 10–50 μg/ml) were tested as single agents as well as drug combinations. To determine the statistical significance of test results, analysis of variance was calculated using the GraphPad Prism software. Data were compared with control experiments using several tumor cell lines and primary tumor cells of patients, calculating synergistic effects by the combination index method using calcusyn software (Biosoft, Cambridge, UK) as described previously [3–5]. The highest *in vitro* response for the induction of apoptosis was achieved with a combination of gemcitabine, oxaliplatin and zoledronic acid (Fig. 2a and b).

Clinical course

According to the results of the *in vitro* chemosensitivity testing, the patient started a combination chemotherapy with a fixed-dose rate regimen of gemcitabine (1000 mg/m^2 as a 10 mg/m^2/min infusion on day 1) and oxaliplatin (100 mg/m^2 as a 2-h infusion on day 2), which was repeated every 2 weeks. Additionally, the patient received a biweekly infusion of the bisphosphonate zoledronic acid (4 mg, day 1). The first cycle of chemotherapy was started on an in-patient basis. Because of rapid improvement of the general performance status, further cycles could be continued on an ambulatory basis.

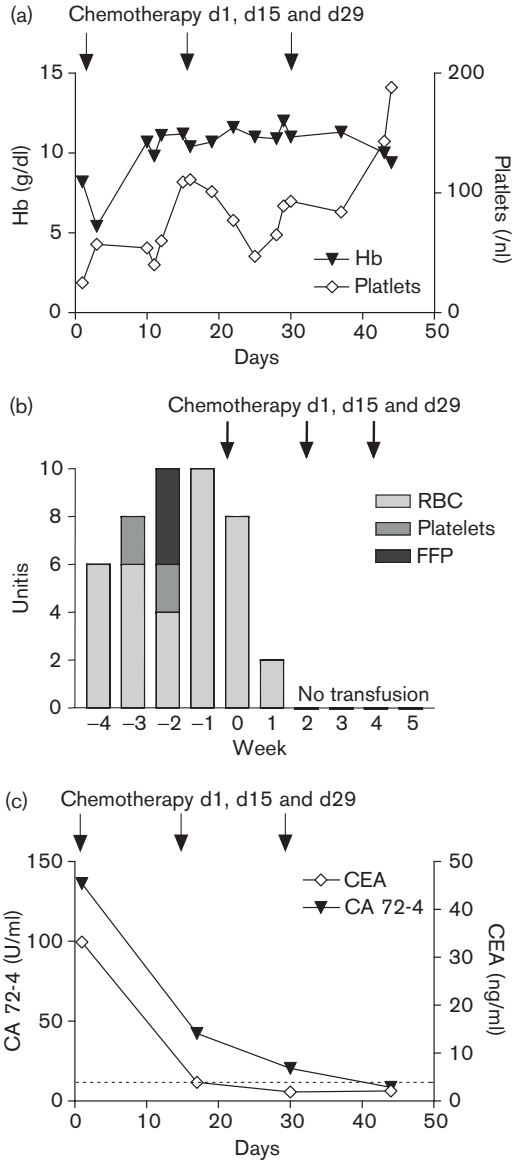
Fig. 2



(a) Range of induction of apoptosis of the drugs oxaliplatin (Ox), cisplatin (Cis), gemcitabine (Gem), doxorubicin (Dox), 5-FU, folinic acid (FA) and zoledronic acid (Zom). Data shown are mean and SE of the percentage of apoptotic cells after 24-h treatment with single drugs, drug combinations containing cisplatin, doxorubicin, 5-FU and folinic acid, and drug combinations of gemcitabine, oxaliplatin and zoledronic acid. (b) Dot-plots of the flow-cytometric analysis of apoptosis. Upper dot-plot shows the rate of apoptosis in the controls, lower dot-blot demonstrates the induction of apoptosis by a gemcitabine + oxaliplatin-based drug combination.

Remarkably, the patient only required blood transfusions in the first 2 weeks after initiation of chemotherapy, thereafter no further transfusions were necessary, and the DIC completely resolved with normalization of thrombocytes, prothrombin time, bilirubin and lactate dehydrogenase levels (Fig. 3a and b). The treatment was tolerated well with minor side-effects including peripheral neuropathy (NCI grade 1) and nausea (NCI grade 1). After 3 months a bone marrow biopsy was repeated, showing only incidental single cells of a signet cell carcinoma. Moreover, the tumor markers CEA and CA72-4 fell impressively to normal levels (Fig. 3c). After 6 months of therapy (10 cycles altogether) no more malignant cells were found in the bone marrow (Fig. 1c), and a repeated local staging by upper-gastrointestinal endoscopy (Fig. 1d) and endoscopic ultrasound revealed scar tissue, which was confirmed histologically. Additionally, no more pathologically enlarged lymph nodes could

Fig. 3



Sequential changes in hemoglobin (Hb) and platelet counts (a), transfusion of red blood cells (RBC), platelets and fresh-frozen plasma (FFP) (b), and levels of the tumor markers carcinoembryonic antigen (CEA) and CA72-4 (c) in a patient with advanced signet cell carcinoma of the stomach and DIC after initiation of combination chemotherapy. The used chemotherapy regimen (zoledronic acid, 4 mg on day 1; gemcitabine, 1000 mg/m^2 as a 10-mg/m^2/min infusion on day 1; oxaliplatin, 100 mg/m^2 as a 2-h infusion on day 2; repeated every 2 weeks) was chosen based on the results of *in vitro* chemosensitivity testing.

be visualized by endoscopic ultrasound or CT scanning at this time. Thus, a complete treatment response according to RECIST criteria was documented. Thereafter, the combination chemotherapy was finished and the patient was followed every 3 months. Currently, after 9 months of follow-up, the patient has no histological and biochemical evidence of recurrence.

Discussion

It would be of major importance to determine appropriate drugs to be used for treatment in patients with advanced malignancy and a very limited life expectancy without intervention. A number of chemosensitivity assays have been developed over the last three decades to predict the responsiveness of tumors to chemotherapy. Early assays proved difficult to use for human tumors, but are widely used with cell lines for drug development [6]. In more recent years, the introduction of newer technology, e.g. the ATP-Tumor Chemosensitivity Assay (ATP-TCA), allowed the performance of valid chemosensitivity testing in solid tumors [7,8]. However, currently available chemosensitivity tests are time consuming and expensive, therefore limiting their widespread use.

In our previous work, we established an *in vitro* chemosensitivity test based on the flow-cytometric monitoring of *in vitro* tumor cell apoptosis in non-Hodgkin's lymphoma using Annexin-V as an early marker of apoptosis [3–5]. Using the same approach in this report, we were able to prove that this test is also able to predict the responsiveness against chemotherapeutic drugs in a patient with a metastasized gastric cancer in less than 24 h. There are some problems limiting the development of flow-cytometric monitoring-based *in vitro* chemosensitivity tests in patients with solid tumors without bone marrow metastases, e.g. contamination with interstitial cells of the primary tumor and technical considerations about how to bring solid tumor cells into a viable suspension [6]. However, the number of tumor cells accessible by bone marrow aspiration in our patient was sufficient to test for *in vitro* chemosensitivity and, additionally, these cells proved ideal for flow-cytometric analysis.

Moreover, we demonstrate that synergistic effects of the combination of gemcitabine and oxaliplatin, which have been reported for patients with advanced pancreatic adenocarcinoma [9], might also translate to signet cell carcinoma of the stomach. The rational for using this combination is further supported by a preclinical study showing synergistic effects of this combination in human leukemia and colorectal cancer cell lines, with an optimal sequence-dependent synergy when tumor cells were exposed to gemcitabine first and then to oxaliplatin 24 h later [10]. It was speculated that synergistic interactions take place at the DNA level and that the incorporation of the antimetabolite gemcitabine into DNA increased oxaliplatin binding to DNA.

A fixed-dose rate regime of gemcitabine was chosen based on its more potent activity, which is attributed to the observation that deoxycytidine kinase is saturated at the plasma gemcitabine concentrations achieved with a standard 30-min infusion, thereby limiting the accumulation of intracellular gemcitabine triphosphate [11]. The

addition of the bisphosphonate zoledronic acid, which has been shown to have direct antiproliferative and apoptotic effects [12], exhibits further synergism.

In summary, the described approach for *in vitro* chemosensitivity testing might be very helpful to predict chemotherapy response in selected patients with metastatic gastric cancer, but also other solid tumors.

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