Circulating Vascular Endothelial Growth Factor and Interferon-γ-inducible Protein-10 Levels in Pancreatic Cancer During Chemotherapy

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Abstract. Background: Chemotherapeutic anticancer properties are thought to derive from apoptosis pathway activation and/or cell division arrest, but animal models have also evidenced anti-angiogenic activity in some agents. Patients and Methods: The impact of gemcitabine, irinotecan and oxaliplatin+5-FU upon the serum markers vascular endothelial growth factor (VEGF) (pro-angiogenic) and IFN-γ-inducible protein (IP)-10 (anti-angiogenic) was evaluated by ELISA in locally advanced and/or metastatic cancer versus clinical efficacy and survival. Results: Patients had higher serum levels of both markers versus controls. No objective response to therapy was observed and no significant difference in either marker occurred during the first month of chemotherapy; analysis by survival showed slight transient VEGF decrease in longer survivors on day 14 and slight increase on day 28 in shorter survivors, who had baseline median IP-10 levels above longer survivors, diverging on day 14 (decrease and increase, respectively). Both groups were below baseline at day 28. Changes in IP-10 were not significant. Conclusion: These preliminary results provide a rationale for exploring whether continuous or frequent administration of some anti-neoplastic agents may elicit a global anti-angiogenic activity, and whether different administration schedules of the same drug could have a synergistic or an antagonistic effect, which obviously would need to be taken into account in determining combinations with new agents targeting angiogenesis.

It has been postulated that angiogenesis, a critical process for tumor growth and progression (1), is regulated by a balance between angiogenic and anti-angiogenic factors (2). Tumor cells may induce angiogenesis directly, via the release of growth factors, and/or indirectly, by attracting inflammatory cells, which in turn release angiogenic stimuli (3). Vascular endothelial growth factor (VEGF) is considered one of the most important vasculogenic mediators (4); its clinical significance in tumor growth is supported by the finding that a number of malignant human tumors, including lung, breast, gastrointestinal tract, ovary and colon (5-9), produce VEGF, and that the inhibition of VEGF-induced angiogenesis significantly inhibits tumor growth in vivo (10).

Chemokines are a large family of chemoattractant cytokines (11) with divergent roles in controlling the growth of malignant tumors. Some chemokines enhance non-specific or specific host immunity against tumor implantation, while others may favor tumor growth and metastasis by promoting tumor cell proliferation or neovascularization in tumor tissues (12). In particular, interferon (IFN)-γ-inducible protein (IP)-10 (CXCL10), a member of the CXC chemokine family that is produced mainly by monocytes, but also by T cells, fibroblasts and endothelial cells, exerts chemotactic activity on lymphoid cells such as T cells, monocytes and NK cells (13). In addition, IP-10 has been shown to inhibit tumor growth in animal models by suppressing neovascularization in antagonism to VEGF (14-16). However, the presence and potential role of the chemokine in clinical tumors remains poorly understood.

The aggressive biology of human pancreatic adenocarcinoma has been linked with VEGF overexpression. Characteristically, the tumor cells are surrounded by a prominent, highly vascularized, desmoplasic stroma and are strongly infiltrated by T cells, neutrophils and macrophages.
Moreover, it has been shown that high VEGF expression predicts early recurrence and poor prognosis after curative resection (18).

It is generally believed that the anticancer properties of chemotherapeutic agents are due to activation of apoptosis pathways and/or arrest of cell division. However, there is evidence that several common anticancer agents, including cyclophosphamide (CTX), doxorubicin (Adriamycin) and paclitaxel (Taxol), have anti-angiogenic activity in animal models (19-21).

Pancreatic cancer is relatively insensitive to most antineoplastic agents. Gemcitabine has replaced 5-fluorouracil (FU)-based therapy, being the most widely accepted first-line therapy for advanced disease, and in phase III trial gave 5.6 months median survival and 18% objective response rate (22). Recent studies have found oxaliplatin plus irinotecan to present activity against gastrointestinal cancer (23-26), and the combination has been suggested as also deserving evaluation in pancreatic cancer, alone or in combination with 5-FU or gemcitabine (24, 25). Gemcitabine, irinotecan and 5-FU have been shown to present anti-angiogenic properties, exercised by means of their action against endothelial cells (26-28). However, in an orthotopic nude mouse model, gemcitabine alone failed to show anti-angiogenic activity on pancreatic carcinoma (29).

The aim of this preliminary study was to evaluate the impact of gemcitabine, irinotecan and a combination of oxaliplatin plus 5-FU upon marker values of serum VEGF and IP-10, in patients with locally advanced and/or metastatic disease. The impact of this regimen upon serum VEGF and IP-10 values in relation to clinical efficacy and survival was preliminarily evaluated.

**Patients and Methods**

**Patient selection.** Patients with advanced, histologically confirmed, pancreatic adenocarcinoma and no prior chemotherapy were enrolled in this study, which was conducted under strict observance of the principles of the Declaration of Helsinki. Other eligibility criteria were measurable or assessable disease not previously irradiated, age >18 years, Zubrod performance status grade ≤2, expected survival >12 weeks, adequate liver, kidney and bone marrow function. Ineligibility included prior malignancy, with the exception of skin basal-cell carcinoma, cervix carcinoma *in situ* or other cancer for which the patient was disease-free for at least 5 years. Other exclusion criteria were prior chemotherapy, radiation therapy within the previous 4 weeks, serious ongoing infection or co-morbidity. Pregnant or breast-feeding women were ineligible, and fertile participants had to agree to practice contraception. All participating patients were required to give informed consent before entering the study.

**Study evaluation and treatment.** The pre-treatment evaluation consisted of a complete medical history and physical examination, blood counts, chemistry profile with hepatic and renal function test and computed tomography scans to document disease extent. The planned accrual was at least three patients per treatment group, as follows: group A: irinotecan 100 mg/m² on days 1, 8 and 15 on a 28-day cycle; group B: oxaliplatin 40 mg/m² and 5-fluorouracil 500 mg/m² as bolus and leucovorin 250 mg/m² on days 1, 8 and 15 on a 28-day cycle; group C: gemcitabine 1000 mg/m² on days 1, 8 and 15 on a 28-day cycle. Thereafter, there was a crossover to each of the other schedules. Toxicity was assessed weekly and adverse events were graded according to the standard National Cancer Institute common toxicity criteria (NCI CTC). Treatment was delayed until recovery in case of hematological toxicity of NCI CTC grade 2 or above. In case of hematological toxicity grade 3 or 4, the subsequent chemotherapy dose was decreased by 25%. Performance status, pain and disease-related symptoms, analgesic consumption and weight (clinical benefit) were recorded at study entry and re-evaluated weekly thereafter. CEA and CA 19.9 were assessed after each course of therapy. A response evaluation was carried out after 3 months of therapy in surviving patients who had a Zubrod performance status ≤2. Peripheral blood samples were collected in clot-activator tubes for VEGF and IP-10 determination from healthy donors, and from patients before the start of therapy and after 2 and 4 weeks. Samples were allowed to clot at room temperature and centrifuged for 10 min at 1900 xg, then stored at −20 °C until analysis.
Determination of VEGF and IP-10 in sera. Circulating VEGF and IP-10 levels were determined by ELISA, using commercially-available Biotrack (Amersham Pharmacia, Little Chalfont, UK) and Cytoscreen (Biosource, Camarillo, CA, USA) kits, respectively. All samples were evaluated in duplicate. The lower detection thresholds were <8 pg/ml and <2 pg/ml, for VEGF and IP-10, respectively.

Statistical analysis. The data are presented as median and range. The Mann-Whitney U-test was used to compare healthy donor, patient baseline and post-chemotherapy levels. Overall survival and progression-free survival were estimated from the start of therapy, with the Kaplan-Meier method of analysis and the 95% Andersen CI.

Results

Between June 2002 and March 2003, 12 patients were enrolled (median age 64 years, range 52-72, 8 m; 4 f). Nine patients had distant metastasis and 3 had locally advanced disease, relapsed after previous surgery.

Before initiating therapy, patients had significantly higher median serum levels of both VEGF and IP-10 versus healthy donors (n=8) (median 294.8 pg/ml, range 149.7-613.1 versus median 185.15 pg/ml, range 117.8-322, p=0.0252 and 139.9 pg/ml, range 49.7-327.3 versus 73.5 pg/ml, range 41.9-154.1, p=0.0259, respectively) (Figure 1).

Four patients started with irinotecan therapy (group A), 3 patients started with oxaplatin and 5-FU (group B) and 5 patients started with gemcitabine (group C). Only 1 of 4 patients in group A completed the 3 sequential schedules, while the remainder only completed the irinotecan course due to sudden clinical deterioration, probably due to progressive disease. All patients in groups B and C completed the 3 sequential schedules, and 2 in group C completed them twice and 3 times, respectively. Because of the high patient drop-out, above all in group A, the analysis of VEGF and IP-10 variation was done only on the samples collected during the first month of chemotherapy.

Median VEGF and IP-10 levels at baseline and at the 14th and 28th day of chemotherapy course in the 12 patients are presented in Table I. No significant difference in either VEGF or IP-10 levels occurred during the first month of chemotherapy.

No objective response to therapy was observed, but 5 patients (1 in group A and 2 each in groups B and C) had stable disease lasting a median of 3.8 months (range 0.9-6.5). Median survival was 3.7 months (range 0.7-21.2), and 6 patients survived beyond 6 months.

As shown in Table II, patient analysis by survival revealed that longer survivors had a slightly transient, though not significant, VEGF decrease over baseline on day 14, whereas shorter survivors had a slight increase on day 28. Relatively shorter survivors had baseline median IP-10 levels higher than those of longer survivors, with a divergent trend on day 14 (decrease in shorter survivors and increase in longer survivors), whereas both groups had a decrease over baseline at day 28. None of the changes in IP-10 reached statistical significance.

Discussion

As angiogenesis is one of the chief parameters determining tumor growth, the effects of chemotherapy on the expression of two well-documented regulators of this process, namely the angiogenic VEGF and the angiostatic IP-10, were investigated. The pancreatic carcinoma patients had significantly higher serum levels of VEGF than the healthy donors, confirming a role for circulating VEGF as an angiogenesis marker. In addition, for the first time, we observed elevated IP-10 serum levels versus normal controls in our small patient group.

While a substantial number of studies have demonstrated a strong association between elevated tumor VEGF expression and advanced disease or poor prognosis in various cancers (30), the loss of endogenous angiostatic molecules has been shown to contribute to tumor-associated angiogenic
activity, enhancing tumorigenesis and spontaneous metastasis (31). Thus, elevated circulating IP-10 levels in patients with advanced or metastatic pancreatic cancer is unexpected and indicative of the complexity of net-tumor-associated neovascularization regulation.

In the 12 cancer patients treated, a transient decrease of VEGF levels occurred after chemotherapy, whereas IP-10 levels were relatively stable. However, in the very small subgroup of patients with a relatively better outcome, during the chemotherapy courses VEGF levels showed a trend towards a more marked decrease on day 14 versus day 28, whereas in the relatively worse outcome subgroup, VEGF levels increased at both times. By contrast, IP-10 levels increased on day 14 only in the relatively better prognosis subgroup, again after the shorter chemotherapy interval. The slight effect found in better-outcome patients on day 14, rather than on day 28, might suggest investigating whether continuous or frequent administration of some antineoplastic agents may elicit a global anti-angiogenic activity, which might be rapidly reversible, and whether different administration schedules of the same drug could have a synergistic or an antagonistic effect, which obviously would need to be taken into account in determining combinations with new agents targeting angiogenesis. In a preclinical model, both anti-angiogenic activity and pro-angiogenic activity have been reported for the same drug, depending on dose and schedule (32). Moreover, in in vitro models, aspirin and morphine treatments have been reported to affect VEGF expression (33, 34). In many cancer patients pain is treated with FANS and opioids, and concurrent therapies might result in a confounding effect. Further studies should focus all these variables.

Because of the small sample size and the interindividual variability in this exploratory study, no estimated value achieved statistical significance during treatment. However, our results do provide questions for further studies.

Acknowledgements

Supported by grants from MIUR (Rome, Italy) (ex-60%) to G.E., and in part by a grant from the Piedmont Regional Government (Regione Piemonte) to G.B.

References


Received January 18, 2005
Revised June 17, 2005
Accepted June 22, 2005