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ORIGINAL ARTICLE

Effect of maximum-tolerated doses and low-dose metronomic chemotherapy on serum vascular endothelial growth factor and thrombospondin-1 levels in patients with advanced nonsmall cell lung cancer

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Abstract
Background Angiogenesis is regulated by a balance of both angiogenic inducers and inhibitors. This study was designed to evaluate the effect of both maximum-tolerated doses (MTD) and low-dose metronomic chemotherapy (LDM) on serum vascular endothelial growth factor (VEGF), thrombospondin-1 (TSP1) and VEGFR1 concentrations in patients with advanced nonsmall cell lung cancer.
Patients and methods Forty consecutive patients with advanced stage nonsmall cell lung cancer were included in this prospective study. Twenty patients received MTD chemotherapy including 75 mg/m^2 of cisplatin and 75 mg/m^2 of docetaxel on day 1. The LDM treatment consisted of cisplatin 25 mg/m^2 and docetaxel 25 mg/m^2 were given to other 20 patients on weeks 1, 2 and 3. Serum levels were prospectively measured in serum by ELISA at four times; before chemotherapy and at 1, 2 and 3 weeks following initiation of chemotherapy.
Results The major finding in this study that MTD chemotherapy but not LDM chemotherapy resulted in significant changes in VEGFR1 and TSP1 serum levels. Due to the effect of LDM chemotherapy, we showed no statistically significant change in patients for all serum VEGF, TSP1 and VEGFR1 levels. Similarly, serum VEGF levels did not also change under MTD chemotherapy. The MTD chemotherapy induced significant and long-lasting increase of TSP1 levels and decrease of VEGFR1 levels that persisted for at least 3 weeks after the chemotherapy initiation. No significant correlations were found between serum VEGF and TSP1 levels in cancer patients treated with both LDM and MTD chemotherapy. The circulating angiogenic balance (TSP1/VEGF) is decreased in cancer patients ($P = 0.039$).
Conclusions The continuous/metronomic chemotherapy may not achieve a more pronounced antiangiogenic effect than MTD-scheduling chemotherapy. Future studies involving a larger number of patients are needed to confirm the present findings.

Keywords Metronomic chemotherapy · MTD chemotherapy · Serum · VEGF · TSP1

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Introduction

Conventional cytotoxic drugs were designed for use at maximum-tolerated doses (MTD) to treat cancer directly by inhibiting or killing rapidly dividing tumor cells [1, 2]. Like many of the rapidly replicating normal host cells that are adversely affected by chemotherapy, tumor endothelial cells are sensitive to chemotherapy. Tumor endothelial cells are different from normal endothelial cells in that they are genetically unstable and their acquired drug resistance is a consequence [2]. However, this is unsubstantiated in the literature. By contrast, endothelial cells in tumor microvessels are, basically genomically stable and therefore less prone to mutate and develop drug-resistance than neoplastic cells, making long term metronomic chemotherapy very attractive also from this important point of view [3–5]. Moreover, it was demonstrated that tumor angiogenesis uses the same signaling pathways as non-tumor angiogenesis [6]. Endothelial cells are damaged by MTD chemotherapy; however, the interruptions between chemotherapy cycles required for patient recovery may allow for tumor regrowth

and endothelial cell repair as well as resulting in an overall reduction of the antiangiogenic effect of the treatment. It clearly demonstrated that antiangiogenic effects of MTD chemotherapy are compromised by the way that chemotherapy is conventionally administered [1, 2].

In an attempt to overcome this, chemotherapy was administered with comparatively low doses of drug on a frequent or continuous schedule, with no extended breaks in order to optimize the antiangiogenic effects of chemotherapy. This approach is referred to as "antiangiogenic chemotherapy" or "low dose metronomic chemotherapy" (LDM) [1, 2]. The activated endothelial cells of newly forming blood vessel capillaries are highly and selectively sensitive to LDM. In other words, unlike MTD, the main targets of which are presumed to be proliferating tumor cells, the main targets of LDM are the endothelial cells of the growing vasculature of a tumor. Therefore, as expectedly, harmful side effects appear to be less severe or even absent treated with LDM chemotherapy [1, 2].

Unlike MTD chemotherapy, antiangiogenic therapy may not result in a measurable decrease in tumor size [1, 2]. For that reason, defining the effective low dose for LDM chemotherapy poses a greater difficulty. An optimal biomarker to assess the effect of antiangiogenic treatment would be noninvasive, applicable to a number of therapeutic options, and provide an early indication of biological activity. There are several classes of markers that could yield useful information. Molecular markers such as circulating levels of endogenous proangiogenic factors [vascular endothelial growth factor (VEGF), bFGF, vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1)] or antiangiogenic factors [thrombospondin-1 (TSP1) and endostatin] also could be used to monitor treatment activity [1, 2, 5]. Of the angiogenic factors studied, VEGF, a surrogate for a proangiogenic stimulus, most reliably predicted tumor growth, whereas TSP1 levels correlate with an antiangiogenic activity of the serum [1, 2, 5, 7].

The mechanisms regulating VEGF-mediated angiogenesis are not well understood. VEGF is a pleiotropic growth factor that mediates multiple functions via its stimulation of cognate receptors on endothelial cells. VEGF and its receptors play a pivotal role in angiogenesis. Activation of the VEGF/VEGF receptor (VEGFR) axis triggers multiple signaling networks that result in endothelial cell survival, mitogenesis, migration, differentiation and vascular permeability. Expression of VEGFR1 and VEGFR2 appears to be upregulated by VEGF. Additionally, TSP1, a well known endogenous inhibitor of angiogenesis, seems to act primarily by binding to CD36 receptors, which are expressed by endothelial cells [1]. This interaction blocks proliferation and induces apoptosis in endothelial cells. TSP can also bind and sequester VEGF, and therefore block its proangiogenic activity [1].

Many of these stimulators and inhibitors of angiogenesis are soluble and diffusible proteins and, therefore, circulating levels could reflect the overall angiogenic activity of the tumor [2]. Measurement of circulating levels of these proteins can be carried out in a relatively noninvasive manner in all cancer patients without biopsy material and the analysis is precise, efficient, and cost effective. Preclinical studies have suggested that monitoring these molecules may have some utility in determining the optimal biological dose.

We attempt to evaluate the effects of both MTD and LDM chemotherapy on serum VEGF, its receptor VEGFR1 and TSP1 concentrations in patients with advanced nonsmall cell lung cancer.

Material and methods

Patients

Between March 2006 and July 2006, 40 consecutive patients (34 males), aged 43–74 years (median age, 59), with advanced, nonresectable stage III ($n = 17$) and metastatic stage IV ($n = 23$) histologically confirmed nonsmall cell lung cancer were included in this prospective study. Eligible patients were required to have measurable or assessable disease, have adequate renal, hepatic and bone marrow functions. The patients were required to have an adequate ECOG performance status (0–2) and life expectancy of at least 3 months. Patients who had received previous cytotoxic treatment for metastatic disease were not enrolled. Written informed consent was obtained from all patients before study. Eighteen normal controls, median age 55 and ranging from 39 to 67 years, were recruited from among the Institute personnel and all were in excellent health at the time of the study.

Treatment

Twenty patients received MTD-based chemotherapy including 75 mg/m^2 of cisplatin and 75 mg/m^2 of docetaxel intravenously as 60-min infusion on day 1. The LDM-type treatment consisted of cisplatin 25 mg/m^2 and docetaxel 25 mg/m^2 given intravenously to other 20 patients on weeks 1, 2 and 3. Treatment cycles were repeated on an outpatient basis every 3 weeks for both groups.

Measurement of serum levels of VEGF, TSP1 and VEGFR1

Blood samples were obtained from patients with advanced stage nonsmall cell lung carcinoma and healthy controls by venipuncture and clotted at room temperature in fasting

conditions on first admission before chemotherapy and 1, 2 and 3 weeks following initiation of chemotherapy. The sera were collected following centrifugation and frozen immediately at −20°C until analysis.

Solid phase-enzyme linked immunosorbent assay (ELISA) was used to determine the serum values of VEGF and VEGFR1 (R & D Systems Inc., Minneapolis, MN, USA) and TSP1 (Chemicon Inc., CA, USA). These assays employ the quantitative sandwich enzyme immunoassay and that according to the manufacturers' method. No significant cross-reactivity or interference was observed. Color developments were proportional to the amount of VEGF, VEGFR1 and TSP1. The color reactions were stopped and the intensity of the colors were measured at 450 nm ELISA reader (Rayto Electronics Inc., China). The measured optical densities were directly proportional to the enzyme concentrations in samples or standards.

Statistical analyses

Data analyses were performed by using SPSS software (SPSS 11, Chicago, IL, USA). The values of serum paramaters were described as median, minimum, and maximum of values. The Mann–Whitney *U* test was used to evaluate differences between patients with lung cancer and normal controls. Basal serum levels were compared with the values determined at 1, 2, and 3 weeks after chemotherapy by using Wilcoxon signed rank test. Spearman's rank correlation tests were used to correlate between different serum parameters. A two-tailed *P* value less than 0.05 was considered statistically significant.

Results

Serum parameters in patients versus controls

We observed tendencies toward higher serum VEGF and lower serum TSP1 values in patients than in healthy subjects, although these differences were not statistically significant ($P = 0.12$, and $P = 0.15$, respectively) (Table 1). However, we found that serum VEGFR1 concentrations in lung cancer patients were significantly higher than in healthy controls ($P < 0.001$).

Modification after chemotherapy usage

Due to effect of LDM based chemotherapy, we showed no statistically significant change in patients with lung cancer for all serum VEGF, TSP1 and VEGFR1 levels ($P > 0.05$) (Table 2).

Similarly, serum VEGF levels did not also change under MTD type chemotherapy (Table 3). But, both TSP1 and VEGFR1 levels are found to be statistically significant changed in lung cancer patients. The MTD based chemotherapy induced significant and long-lasting increase of TSP1 levels and decrease of VEGFR1 levels that persisted to at least 3 weeks after the chemotherapy application.

We did not show any correlation between the changes in levels of circulating parameters and tumor response to chemotherapy.

Correlations of serum parameters

No significant correlations were found between serum VEGF and TSP1 levels in cancer patients treated with both LDM and MTD type chemotherapy (Table 4). However, under LDM type treatment, we determined significant correlations between both serum VEGF and VEGFR1 and serum TSP1 and VEGFR1 levels.

Angiogenic balance

Because the angiogenesis process is controlled by the balance between factors that promote and inhibit angiogenesis, we studied the circulating angiogenic balance (TSP1/VEGF). Serum TSP1/VEGF value in the control group is significantly higher than in patients ($P = 0.039$). Similar results were obtained in patients treated with chemotherapy of neither LDM nor MTD bases.

Discussion

The development of a noninvasive test for assessment of angiogenic activity is critical in era of targeted therapies [7]. This would mean determining the mechanism of action of antiangiogenesis-targeted therapies. With this rationale, we organized to examine the feasibility of testing serum

Table 1 Distribution of basal serum levels of VEGF, TSP1 and VEGFR1 in patients with lung cancer and healthy controls

Parameter	Patients ($n = 40$)		Controls ($n = 18$)		*P*-value
	Median value	Range	Median value	Range	
VEGF (pg/ml)	265.8	55.8–1379.7	188.9	4.8–474.0	0.122
TSP1 (ng/ml)	176775.8	96596.9–205790.6	180464.8	110115.7–201608.8	0.158
VEGFR1 (ng/ml)	0.812	0.622–2.518	0.666	0.477–0.982	<0.001

Table 2 Modifications of angiogenesis parameters after LDM chemotherapy

Parameter	Median	Range	Percentage change vs baseline	*P*
VEGF (pg/ml)				
Basal level	265.8	55.8–1379.7	–	
1 week	227.3	35.8–1455.8	−0.973	0.823
2 weeks	235.3	0.3–1615.0	−3.298	0.737
3 weeks	228.5	6.3–1308.8	−6.513	0.472
TSP1 (ng/ml)				
Basal level	176775.8	96596.9–205790.6	–	
1 week	173437.5	26067.6–215775.3	−2.888	0.370
2 weeks	187689.2	86835.3–212712.1	3.587	0.370
3 weeks	185388.3	9191.5–206147.3	1.465	0.744
VEGFR1 (ng/ml)				
Basal level	0.812	0.622–2.518	–	
1 week	0.870	0.456–2.246	1.108	0.627
2 weeks	0.854	0.538–1.638	5.198	0.970
3 weeks	0.831	0.640–1.666	3.614	0.723

Table 3 Modifications of angiogenesis parameters after MTD chemotherapy

Parameter	Median	Range	Percentage change vs baseline	*P*
VEGF (pg/ml)				
Basal level	280.2	6.2–1894.1	–	
1 week	204.0	5.9–1281.3	−6.605	0.370
2 weeks	334.8	3.9–2094.2	−16.848	0.433
3 weeks	364.8	5.5–1167.9	42.241	0.135
TSP1 (ng/ml)				
Basal level	145085.3	11439.2–202994.0	–	
1 week	148152.2	29020.0–200081.0	7.947	0.126
2 weeks	175914.9	98622.5–203598.1	17.119	**0.021**
3 weeks	169984.9	1482.9–205847.3	14.265	**0.030**
VEGFR1 (ng/ml)				
Basal level	0.822	0.584–2.202	–	
1 week	0.787	0.438–2.346	−1.583	0.823
2 weeks	0.770	0.458–1.128	−10.056	**0.052**
3 weeks	0.791	0.356–1.172	−23.634	**0.040**

Table 4 Correlation between serum parameter levels at different time points

Time points	VEGF vs TSP1		VEGF vs VEGFR1		TSP1 vs VEGFR1	
	LDM (*P*)	MTD (*P*)	LDM (*P*)	MTD (*P*)	LDM (*P*)	MTD (*P*)
Basal level	NS	NS	NS	NS	NS	NS
1 week	NS	NS	NS	NS	0.032 ($r = -0.479$)	NS
2 weeks	NS	NS	0.042 ($r = 0.459$)	NS	NS	NS
3 weeks	NS	NS	0.056 ($r = -0.458$)	NS	0.001 ($r = -0.732$)	NS

NS nonsignificant

levels of angiogenesis parameters as a surrogate marker of lung cancer angiogenesis.

In the present study we found that our patients with lung carcinoma had higher serum levels of VEGF than healthy persons, although the differences did not reach statistical significance. This finding has been previously reported by several authors [3–10]. Likewise, although the difference for TSP1 was not significant, our results show that serum

concentrations were lower in patients than in control subjects. This finding may be consistent with the hypothesis that TSP1 levels would decrease during the initiation of tumor angiogenesis and in patients with advanced disease. For this subject, studies with different findings such as decreased [7] and increased [11] serum levels of TSP1 in lung cancer are found in literature.

Our results show a decrease in the angiogenesis balance defined as TSP1/VEGF in patients as compared to healthy controls, as also reported by Gonzalez et al. [12]. This finding may shift the balance toward a proangiogenic profile in cancer patients, and thus lead to an increase in angiogenic activity and disease progression [12].

Due to effect of LDM chemotherapy, we showed no statistically significant change in patients with lung cancer for all serum VEGF, TSP1 and VEGFR1 levels. Similarly, serum VEGF levels did not also change under MTD chemotherapy. However, MTD chemotherapy induced significant and long-lasting increase of TSP1 levels and decrease of VEGFR1 levels that persisted to at least 3 weeks after the chemotherapy application. Dudek et al. [7] found that 1 week following MTD-based chemotherapy serum VEGF levels significantly dropped in almost all patients. However, at 12 weeks after the initiation of chemotherapy, in nonsmall cell lung cancer patients who responded the therapy, serum VEGF levels remained below their pretreatment levels. Additionally, serum levels of TSP1 were not affected and changed by MTD-style chemotherapy [7]. Damber et al. [13], found that systemic low-dose continuous treatment of a rat prostate cancer model with cyclophosphamide and paclitaxel induced the expression of TSP1 in tumor tissue and inhibited tumor growth. They supposed that antitumor effect of low-dose metronomic chemotherapy, at least with certain chemotherapeutics, is partly mediated by induction of endogenous anti-angiogenic factors. Likewise, Bocci et al. [14] showed that mice treated with LDM cyclophosphamide had increased levels of circulating TSP1. Hamano et al. [15] determined intratumoral expression of TSP1 is increased in tumors treated with LDM chemotherapy.

In conclusion, to our knowledge, this is the first report of such finding in adult human patients on clinical basis. We found that continuous/metronomic chemotherapy might not achieve a more pronounced antiangiogenic effect than MTD-scheduling chemotherapy. Future studies involving a larger number of patients are needed to confirm the present findings.

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