CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS
Volume 27, Number 3, 2012

DOI: 10.1089/cbr.2011.1103

Low-Dose Docetaxel Combined with (−)-Epigallocatechin-3-Gallate Inhibits Angiogenesis and Tumor Growth in Nude Mice with Gastric Cancer Xenografts

Hongju Wu, Yan Xin, Yuping Xiao, and Jing Zhao

Abstract

Low-dose metronomic (LDM) chemotherapy represents a new strategy to treat solid tumors by stronger antiangiogenic activity and less side-effects, especially in combination with other antiangiogenic agents. The aim of the study is to investigate the antiangiogenic effect of docetaxel alone and combined with (−)-epigallocatechin-3-gallate (EGCG) in preclinical settings of gastric cancer. BGC-823 human gastric cancer xenograft model was used, and tumor growth, side-effects of mice were closely monitored. Expression of vascular endothelial growth factor and CD31 were observed by immunohistochemistry, and microvessel density of the tumor tissues was assessed by CD31 immunohistochemical analysis. Our results indicated that LDM docetaxel inhibited angiogenesis and growth of gastric cancer with less toxicity, and the effects were further enhanced by the concurrent administration of EGCG. Our study, for the first time, rationally demonstrated that LDM docetaxel treatment used alone or combined with EGCG is effective and safe in preclinical settings of gastric cancer. Our data suggest that LDM docetaxel used alone or combined with EGCG may be an innovative and promising therapeutic strategy in the experimental treatment of human gastric cancer.

Key words: angiogenesis, docetaxel, EGCG, gastric cancer, metronomic chemotherapy

Introduction

Despite the progress in cancer therapeutics and chemotherapy development with the introduction of new drugs, advanced gastric cancer continues to have an extremely poor prognosis and with limited treatment options.[1] Thus, new therapeutic strategies are urgently needed. Antiangiogenic therapy is one of the most promising novel strategies and many attempts have been made to prevent or delay tumor growth by antiangiogenesis. Recently, there has been considerable interest in the notion of exploiting chemotherapeutic drugs as angiogenesis inhibitors. Conventional application of chemotherapy, directly targeting the tumor cells, is based on the linear dose–efficacy relationship for these drugs resulting in a cyclic treatment to allow for recovery from the side-effects. Any potential damage to the tumor vasculature can be repaired during the long breaks. Continuous, low-dose metronomic (LDM) chemotherapy, directly targeting the endothelial cells is thought to have fewer side-effects and a lack of drug resistance.[2] And metronomic chemotherapy is changing the paradigm that more is better.[3]

Docetaxel is a mitotic spindle poison that induces a mitotic block, and exhibits promising activity in gastric cancer.[4] Chemotherapeutic drugs, and docetaxel in particular, chronically administered using a frequent schedule at low dose (metronomic dosing), can cause potent antiangiogenic effects by targeting the endothelial cells of newly growing blood vessels.[5] The effects of LDM chemotherapy regimens can also be improved by concurrent administration of a drug-targeting angiogenesis.[6,7] The rationale for this combination is that these targeted drugs capable of specific blockade of activated endothelial cell survival mechanisms, will selectively enhance the damaging or cytotoxic effects of LDM chemotherapy on newly formed blood vessels.[8]

Fourth Laboratory of Cancer Institute, Department of Tumor Pathology of General Surgery Institute, No.1 Hospital of China Medical University, Shenyang, Liaoning Province, China.

Address correspondence to: Yan Xin; Fourth Laboratory of Cancer Institute, Department of Tumor Pathology of General Surgery Institute, No. 1 Hospital of China Medical University; 155 Nanjing North Street, Heping District, Shenyang 110001, Liaoning Province, China
E-mail: **xinyan9988@hotmail.com**

Naturally occurring substances that are derived from diet provides a new insight in cancer therapy. Tea is the most widely consumed beverage worldwide. Green tea extract and its major component (−)-epigallocatechin-3-gallate (EGCG) possess obvious antiproliferative, proapoptotic, and antiangiogenic effects.[9] EGCG is an agent, which, exhibits antiangiogenic activities through a variety of mechanisms.[10,11] Several studies have suggested that EGCG may reduce the toxicity of certain anticancer drugs. These studies suggest that EGCG could be used in adjuvant settings for cancer management.[9]

Our previous study[12] proved docetaxel had a significant decrease in IC_{50} values of endothelial cells compared with BGC-823 gastric cancer cells *in vitro*, indicating antiangiogenic doses are far below the optimal doses for direct antitumor activity. This study evaluated the combination of low-dose docetaxel with EGCG in gastric cancer treatment. The aims of this research were (1) to compare the antitumor and antiangiogenic effects of LDM docetaxel with the maximum tolerated dose (MTD) and (2) to determine the feasibility of combining LDM docetaxel therapy with EGCG for the treatment of gastric cancer and also to verify its antiangiogenic mechanism.

Materials and Methods

Materials

Human gastric cancer cell line BGC-823 was obtained from the Cancer Research Institution of China Medical University. A group of female BALB/c nude mice (6–8 week age) weighing between 18 and 20 g were purchased from the Animal Experiment Center of Beijing. Mice were housed under pathogen-free conditions, and fed with animal chow and water *ad libitum*. Docetaxel was supplied by Jiangsu Hengrui Medicine Company. EGCG was obtained from Shanghai Winherb Medical Company. Human monoclonal antibody for vascular endothelial growth factor (VEGF) was purchased from Fuzhou Maixin Company. Mouse monoclonal antibody for CD31 was purchased from Dako.

Cell culture

Human BGC-823 gastric cancer cells were cultured in Dulbecco's modified Eagle's medium (RPMI 1640) routinely supplemented with 10% fetal bovine serum plus ampicillin and streptomycin, and incubated in 5% CO_2 at 37°C.

Design of animal experiments

All the animal experiments were approved by the Institutional Animal Care and Use Committee of China Medical University. BGC-823 Cells (2×10^6) were suspended in 0.2 mL of phosphate-buffered saline (PBS) and injected into the right flank of each BALB/c nude mice. Approximately 2 weeks later (average tumor size, 100 mm^3), the mice were randomized into five groups (each group had 10 mice) as follows: control (intraperitoneal [i.p.] injection of 0.2 mL physiological saline daily]; MTD (i.p. injection of 15 mg/kg docetaxel once every 2 weeks); LDM (i.p. injection of 0.5 mg/kg docetaxel three times a week); EGCG (i.p. injection 1.5 mg EGCG daily) and the combined therapy with LDM docetaxel and EGCG at doses identical to the single agent. The respective doses of MTD for docetaxel and the optimal metronomic dose for docetaxel were selected as they had been previously reported in mice *in vivo*.[13,14] In addition, the dose and route of administration of EGCG was based on previous studies[15,16] which demonstrated that EGCG (1.5 mg, i.p.) had significant antiamgiogenic effects and reduced VEGF production in mice *in vivo*.

Tumor growth and side-effects

The mice were closely monitored and body weight and tumor size recorded once a week. Tumor volume (TV) was estimated using the formula: TV (mm^3) = (width$^2\times$length)/2. Blood samples were collected through orbital sinus once a week to count white blood cells (WBC). During the experiment period, side-effects such as weight loss, change in behavior and feeding, reaction to stimulation, and ruffling of fur were observed. Moribund mice were euthanized according to the pre-established criteria, namely the presence of one or more of the following premorbid conditions: gross ascites, palpable tumor burden greater than 2000 mm^3, dehydration, lethargy, or weight loss greater than 20% of initial body weight. If more than half of the mice in one group had shown above circumstances, all the mice in this group were killed by an anesthetic overdose. The experimental period ended 56 days after the inoculation of tumor cells. Tumors were excised and tumor tissue samples from all the different treatment groups were fixed in 10% phosphate-buffered formaldehyde for 12–24 hours and embedded in paraffin for histology and immunohistochemistry.

Immunohistochemical detection of VEGF and CD31

Five-micrometer sections were cut from paraffin blocks and were stained with hematoxylin–eosin for histological analysis. Adjacent sections were cut for immunohistochemistry using the PV-9000 kit (Beijing Zhongshan Goldenbridge Biotechnology Company). Rabbit anti-human monoclonal antibody for VEGF was diluted for 1:50 and rat anti-mouse monoclonal antibody for CD31 was diluted for 1:100. Antigens were retrieved after they were placed in a pressure cooker at full pressure for 160 seconds in citrate buffer (pH 6.0). All procedures were implemented according to their manufacturer's instructions, respectively. For negative controls, sections were processed as above but treated with 0.01 mol/L PBS instead of primary antibodies.

Cytoplasmic staining was scored positive for VEGF. The degree of positivity was evaluated by calculating the percentage of immunoreactive cells on a minimum of 500 cells.[17]

Microvessel density (MVD) was assessed by immunohistochemical analysis with antibodies to the endothelial marker CD31 and determined according to the method of Liu TG and colleagues.[18] Briefly, the immunostained sections were initially screened at low magnifications (40× and 100×) to identify hot spots, which are the areas of highest neovascularization. Any yellow brown stained endothelial cell or endothelial cell cluster that was clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements was considered a single, countable microvessel. Within the hot spot area, the stained microvessels were counted in a single high-power (200×) field, and the average vessel count in three hot spots was considered the value of MVD. All counts were performed by three investigators in a blinded manner. Microvessel counts were compared between the

observers and discrepant results were reassessed. The consensus was used as the final score for analysis.

Statistical analysis

Data were analyzed by ANOVA, followed by the Student–Newman–Keuls test. All statistical analyses were performed by using SPSS 17.0 software package. Data were expressed as the mean values ± standard error. p-Values lower than 0.05 were considered significant.

Results

Tumor-growth assessment

As shown in Figure 1, the tumors of more than half of the mice in the control group were greater than 2000 mm^3 42 days after implantation, thus all the mice in this group were killed according to the pre-established criteria. In contrast, in the MTD and LDM groups, the tumors had reached only 1234 ± 125 and 775 ± 98 mm^3 42 days after implantation, respectively, being significantly smaller than the control tumors. Interestingly, when LDM docetaxel was continuously administered, tumor growth was delayed, compared with the MTD group, when assessed after 2 weeks of treatment (*$p < 0.05$; Fig. 1). EGCG retarded tumor growth to much the same extent as MTD docetaxel. Combined therapy with metronomic docetaxel and EGCG resulted in tumor-growth delays that were more remarkable and enduring than those resulting from either of them when used alone (**$p < 0.05$; Fig. 1).

Toxicity evaluation

Mice treated with the conventional MTD regimen appeared to be reluctant to move, and their skin was flabby and lackluster. The five groups of mice had similar WBC counts (Fig. 2) and body weights (Fig. 3) in the beginning, but 2 weeks after initiation of antitumor therapy, obvious decreases of WBC counts and body weights were observed in the MTD group (both *$p < 0.05$). The continuous LDM docetaxel regimen, EGCG regimen and the combined therapy with LDM docetaxel and EGCG were not associated with weight loss, leucopenia, or other signs of toxicity.

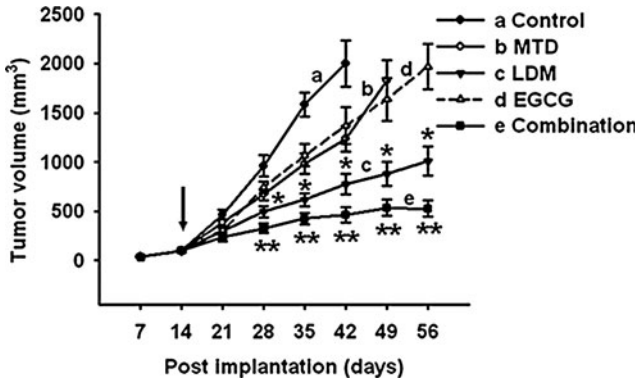

FIG. 1. Tumor-growth curve of each group. BGC-823 gastric tumors were established in BALB/c nude mice. Two weeks later, when the size of tumors reached around 100 mm^3 (indicated by a vertical arrow), they were assigned into five groups: Control, MTD, LDM, EGCG, and Combination (LDM + EGCG). After 2 weeks of treatment, tumor growth was delayed in the LDM group compared with the MTD group (*$p < 0.05$); EGCG retarded tumor growth to much the same extent as MTD docetaxel; when LDM docetaxel was combined with EGCG, tumor growth delays were more remarkable and enduring than those resulting from either of them when used alone (**$p < 0.05$). *$p < 0.05$ versus MTD group; **$p < 0.05$ versus LDM and EGCG groups. EGCG, (−)-epigallocatechin-3-gallate; LDM, low-dose metronomic; MTD, maximum tolerated dose.

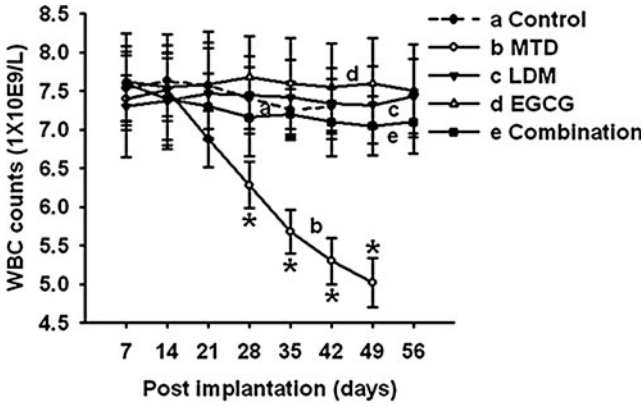

FIG. 2. WBC counts of BALB/c nude mice in each group. The five groups of mice had a similar WBC counts in the beginning, but 2 weeks after initiation of antitumor therapy, conventional MTD chemotherapy produced significant leucopenia compared with the other four groups. *$p < 0.05$ versus other groups. WBC, white blood cells.

Histopathologic analysis

A comparison of angiogenic indices revealed some variance among different groups of treatment. Tumors derived from mice in the control group (Fig. 4A) showed the highest microvessel counts (26.93 ± 2.05)/200× field, whereas those from mice subjected to MTD group (Fig. 4B) had comparatively lower microvessel counts (17.82 ± 2.02)/200× field ($^a p < 0.05$, Fig. 5). LDM group (Fig. 4C) or EGCG group (Fig. 4D) had lower microvessel counts than MTD group

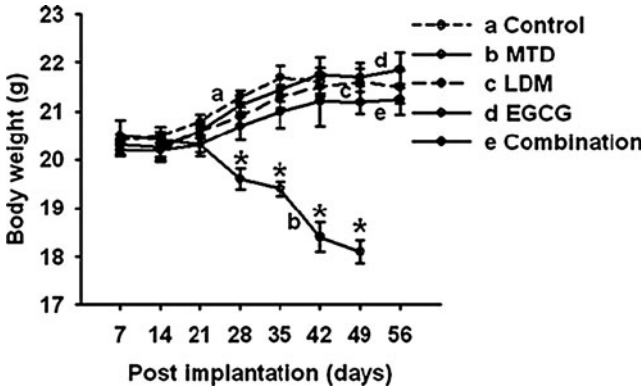

FIG. 3. Body weights of BALB/c nude mice in each group. The five groups of mice had similar body weights in the beginning, but 2 weeks after initiation of antitumor therapy, conventional MTD chemotherapy produced significant weight loss compared with the other four groups. *$p < 0.05$ versus other groups.

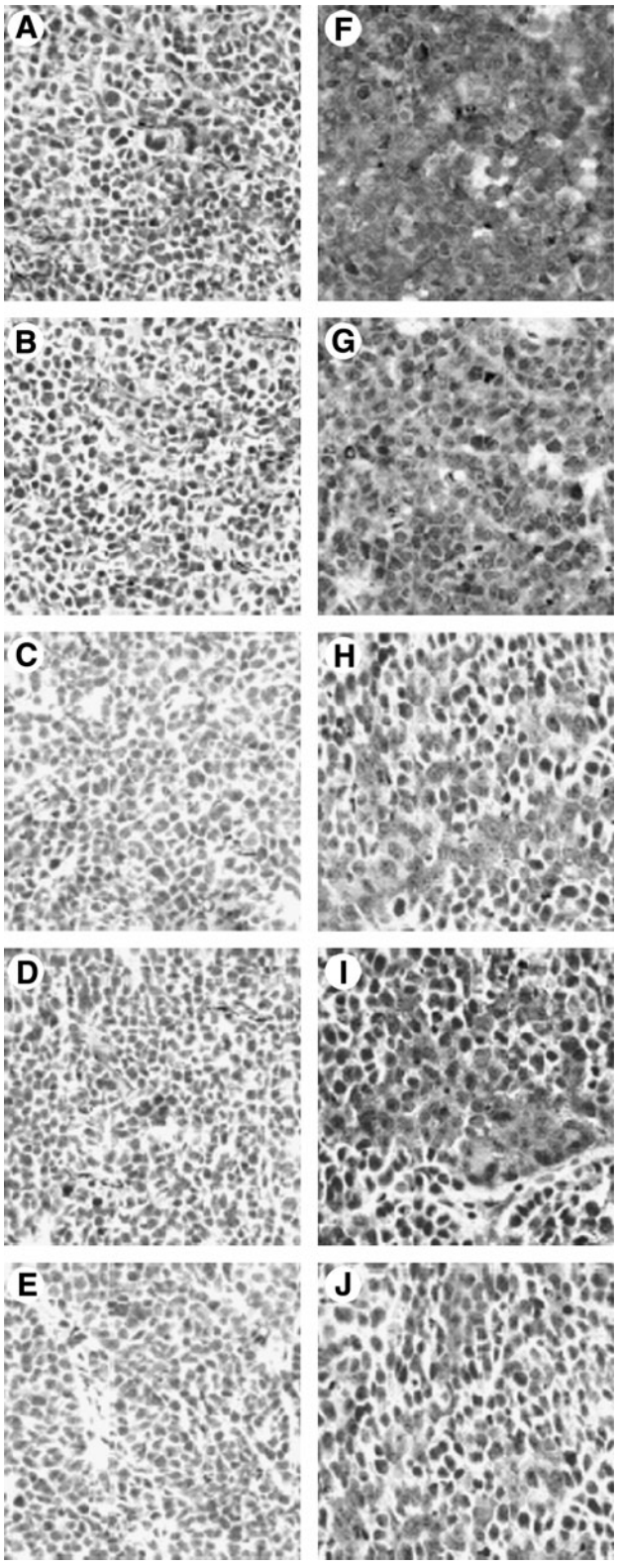

FIG. 4. Representative images of immunohistochemistry of mouse CD31 and human VEGF in BGC-823 xenografts. **(A–E)** Illustrated are immunohistochemical staining of CD31 (200×). **(A)** control group; **(B)** MTD group; **(C)** LDM group; **(D)** EGCG group; **(E)** combination group. **(F–J)** Illustrated are immunohistochemical staining of VEGF (200×). **(F)** control group; **(G)** MTD group; **(H)** LDM group; **(I)** EGCG group; **(J)** combination group. VEGF, vascular endothelial growth factor.

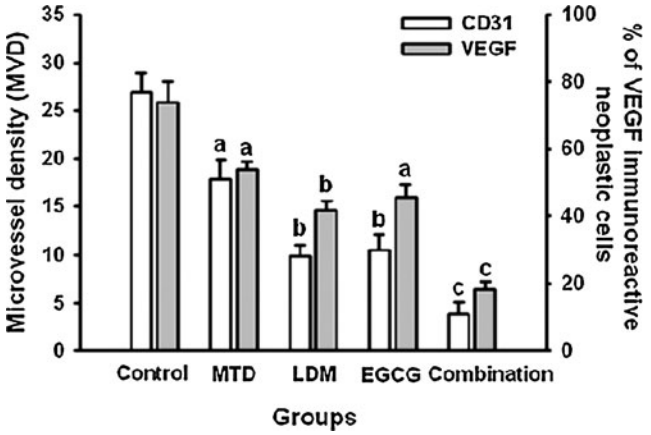

FIG. 5. Quantification of MVD and VEGF positivity in BGC-823 tumor xenografts. Columns and bars, mean values ± standard error, respectively. $^{a}p < 0.05$ versus control group; $^{b}p < 0.05$ versus MTD group; $^{c}p < 0.05$ versus LDM and EGCG groups. MVD, microvessel density.

($^{b}p < 0.05$, Fig. 5). However, the lowest counts [(3.79 ± 1.87)/200× field] in combination group (Fig. 4E) were measured ($^{c}p < 0.05$, Fig. 5). Analysis of vessel morphology showed that tumors with combined treatment were characterized by small capillaries and lacked the large telangiectatic vessels or glomeruloid structures seen in tumors from the control group or from mice subjected to conventional chemotherapy. There was positive expression of VEGF in the cytoplasm of some tumor cells. Compared with the MTD group (Fig. 4G), VEGF expression was lower in the continuous LDM docetaxel group (Fig. 4H) ($^{b}p < 0.05$, Fig. 5). When EGCG was added to continuous LDM docetaxel treatment (Fig. 4J), the indicators were much lower ($^{c}p < 0.05$, Fig. 5). The results indicated that metronomic docetaxel chemotherapy inhibited tumor angiogenesis and its antiangiogenic effect was further improved when combined with EGCG.

Discussion

Our translational study, for the first time, rationally demonstrated that LDM docetaxel used alone or combined with EGCG is effective in preclinical settings of gastric cancer, as an antiangiogenic and antitumor schedule, modulating VEGF gene expression.

The evolution toward metronomic administration of chemotherapeutic drugs is based on several factors: (1) high-dose chemotherapy is not very effective and is associated with high toxicity; (2) metronomic chemotherapy may significantly delay the onset of mutation-dependent mechanisms of acquired drug resistance; (3) metronomic chemotherapy, in which the cumulative dose is significantly less than MTD-based chemotherapy, has several potential advantages, including lower toxicity and adverse side-effects; (4) more importantly, it seems that despite the lower cumulative doses administered, metronomic chemotherapy is superior to MTD-based regimens for inhibiting tumor growth in preclinical models and clinical trials.[5,18,19] Our findings show that metronomic docetaxel had significant effects on therapeutic response. One of the major factors that account for the limited advances made in cancer treatment is

acquired drug resistance. Metronomic chemotherapy is less likely to acquire resistance compared with conventional chemotherapy, since the target of the therapy is presumed to be the genetically stable and activated endothelial cell rather than the genetically unstable cancer cell.[20] In the present study, tumor growth was delayed at the prophase in mice receiving MTD docetaxel, after which the tumors began to grow progressively while on therapy, which could be easily explained by drug resistance. But when LDM docetaxel was administered, the regimen produced a long period of growth delay in tumor mass.

An attractive application of metronomic chemotherapy is the ability to combine these regimens with biological agents, in particular antiangiogenic drugs and the effects of these schedules may be further enhanced.[21,22] Such combinations are particularly appealing because high local concentrations of VEGF in the tumor environment can promote multidrug resistance in tumor endothelium.[6,23,24] In our study, metronomic docetaxel plus EGCG led to a substantial tumor reduction over the respective monotherapies. Moreover, we did not observe toxic effects at the doses administered in the LDM and combinational groups, as evidenced by body weight and WBC counts, which remained similar to those of control mice during the treatment period.

Angiogenesis plays an important role in both tumor growth and metastasis.[25] Angiogenesis is tightly regulated by pro-angiogenic and antiendothelial growth factors. VEGF is one of the most essential pro-angiogenic growth factors,[26] and it also appears to be critical in the angiogenic process.[27] MVD is accepted as a standard indicator of angiogenesis,[28] and VEGF expression is strictly correlated with MVD.[29] In the present study, MVD in tumor xenografts was markedly decreased in metronomic schedules when compared with control group and also to the MTD regimen, which were in agreement with the findings of Bocci, et al.[7,21,30] In addition, MVD in the combination group significantly decreased compared with the LDM and EGCG groups. VEGF protein expression in tumors was similar to that of MVD. Our data indicated that LDM chemotherapy exhibited the inhibitory effects of angiogenesis by decreasing MVD value and VEGF expression. And the effects were improved by concurrent administration of EGCG.

Altogether, data above indicate that LDM docetaxel and EGCG may potentiate each other's antitumor and antiangiogenesis activities. The mechanism responsible for the interaction between LDM docetaxel and EGCG remains unclear. On one hand, EGCG can modulate the expression of the multidrug resistance proteins.[31] Moreover, EGCG and its metabolites are substrates for drug efflux pumps and can compete with chemotherapeutic drugs.[32,33] By saturating these efflux mechanisms, the effect of chemotherapeutic drugs can be increased. On the other hand, EGCG inhibited angiogenesis by blocking Erk-1 and Erk-2 activation and VEGF expression.[15] EGCG also inhibited VEGF-induced endothelial cell proliferation, migration, and tube formation.[16] In addition, EGCG has been shown to modulate growth-factor signaling pathways and, additionally, has effects on cell-cycle progression and tumor cell invasion.[34] The above effects may contribute to the synergistic inhibition of tumor angiogenesis and growth. However, the exact mechanisms need further study.

In summary, the results presented here have shown an effective and safer strategy by using LDM docetaxel chemotherapy alone or by combining it with EGCG, compared with MTD docetaxel therapy in tumor growth and side-effects, in a nude mouse model of BGC-823 gastric cancers. Our data suggest that LDM docetaxel used alone or combined with EGCG is highly efficacious and should be considered for clinical trials and further preclinical studies.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (No. 30973503, No. 81071650, and No. 81050007), Special foundation for Climbing Scholars of Universities in Liaoning Province (2009–2011) and Research Fund for the Doctoral Program of Higher Education (20092104110008).

Disclosure Statement

No competing financial interests exist.

References

1. Albert A. New drugs in the treatment of gastric tumors. Clin Transl Oncol 2008;10:256.
2. Orlando L, Cardillo A, Rocca A, et al. Prolonged clinical benefit with metronomic chemotherapy in patients with metastatic breast cancer. Anticancer Drugs 2006;17:961.
3. Scharovsky OG, Mainetti LE, Rozados VR. Metronomic chemotherapy is changing the paradigm that more is better. Curr Oncol 2009;16:7.
4. Tetzlaff ED, Cheng JD, Ajani JA. Review of docetaxel in the treatment of gastric cancer. Ther Clin Risk Manag 2008; 4:999.
5. Benelli R, Monteghirfo S, Balbi C, et al. Novel antivascular efficacy of metronomic docetaxel therapy in prostate cancer: hnRNP K as a player. Int J Cancer 2009;124:2989.
6. Bergers G, Hanahan D. Combining antiangiogenic agents with metronomic chemotherapy enhances efficacy against late-stage pancreatic islet carcinomas in mice. Cold Spring Harb Symp Quant Biol 2002;67:293.
7. Garcia AA, Hirte H, Fleming G, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: A trial of the California, Chicago, and princess Margaret hospital phase II consortia. J Clin Oncol 2008;26:76.
8. Klement G, Baruchel S, Rak J, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 2000;105:R15.
9. Khan N, Afaq F, Saleem M, et al. Targeting multiple signaling pathways by green tea polyphenol (-) -epigallocatechin-3-gallate. Cancer Res 2006;66:2500.
10. Neuhaus T, Pabst S, Stier S, et al. Inhibition of the vascular-endothelial growth factor-induced intracellular signaling and mitogenesis of human endothelial cells by epigallocatechin-3 gallate. Eur J Pharmacol 2004;483:223.
11. Fassina G, Vene R, Morini M, et al. Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. Clin Cancer Res 2004;10:4865.
12. Hongju Wu, Yan Xin, Jing Zhao, et al. Metronomic docetaxel chemotherapy inhibits angiogenesis and tumor growth in a gastric cancer model. Cancer Chemother Pharmacol 2011;68:879.

13. Dykes DJ, Bissery MC, Harrison SD, et al. Response of human tumor xenografts in athymic nude mice to docetaxel (RP 56976, Taxotere). Invest New Drugs 1995;13:1.
14. Kamat AA, Kim TJ, Landen CN, et al. Metronomic chemotherapy enhances the efficacy of antivascular therapy in ovarian cancer. Cancer Res 2007;67:281.
15. Jung YD, Kim MS, Shin BA, et al. EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br J Cancer 2001;84:844.
16. Zhu BH, Zhan WH, Li ZR, et al. (-)-Epigallocatechin-3-gallate inhibits growth of gastric cancer by reducing VEGF production and angiogenesis. World J Gastroenterol 2007; 13:1162.
17. Bocci G, Falcone A, Fioravanti A, et al. Antiangiogenic and anticolorectal cancer effects of metronomic irinotecan chemotherapy alone and in combination with semaxinib. Br J Cancer 2008;98:1619.
18. Liu TG, Huang Y, Cui D, et al. Inhibitory effect of ginsenoside Rg3 combined with gemcitabine on angiogenesis and growth of lung cancer in mice statistical analysis. BMC Cancer 2009;9:250.
19. Kerbel RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004;4:423.
20. Bocci G, Nicolaou KC, Kerbel RS. Protracted low-dose effects on human endothelial cell proliferation and survival *in vitro* reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res 2002;62:6938.
21. Zhang M, Tao W, Pan S, et al. Low-dose metronomic chemotherapy of paclitaxel synergizes with cetuximab to suppress human colon cancer xenografts. Anticancer Drugs 2009;20:355.
22. Zhang Q, Kang X, Zhao W. Antiangiogenic effect of low-dose cyclophosphamide combined with ginsenoside Rg3 on Lewis lung carcinoma. Biochem Biophys Res Commun 2006;342:824.
23. Kerbel RS. Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anticancer therapeutic agents. Bioessays 1991;13:31.
24. Castilla MA, Caramelo C, Gazapo RM, et al. Role of vascular endothelial growth factor (VEGF) in endothelial cell protection against cytotoxic agents. Life Sci 2000;67:1003.
25. Folkman J. What is the evidence that tumors are angiogenesis dependent. J Natl Cancer Inst 1990;82:4.
26. Bando H. Vascular endothelial growth factor and bevacitumab in breast cancer. Breast Cancer 2007;14:163.
27. Hayes DF, Miller K, Sledge G. Angiogenesis as targeted breast cancer therapy. Breast 2007;16(supp2):S17.
28. Magnon C, Galaup A, Rouffiac V, et al. Dynamic assessment of antiangiogenic therapy by monitoring both tumoral vascularization and tissue degeneration. Gene Ther 2007;14:108.
29. Maria RR, Francesca C, Francesca G. Microdensity vessels and with vascular endothelial growth factor expression in ovarian carcinoma. Int J Surg Pathol 2005;13:135.
30. Ji Y, Hayashi K, Amoh Y, et al. The camptothecin derivative CPT-11 inhibits angiogenesis in a dual-color imageable orthotopic metastatic nude mouse model of human colon cancer. Anticancer Res 2007;27:713.
31. Mei Y, Qian F, Wei D, et al. Reversal of cancer multidrug resistance by green tea polyphenols. J Pharm Pharmacol 2004;56:1307.
32. Hong J, Lambert JD, Lee SH, et al. Involvement of multidrug resistance-associated proteins in regulating cellular levels of (-)-epigallocatechin-3-gallate and its methyl metabolites. Biochem Biophys Res Commun 2003;310:222.
33. Hong J, Lu H, Meng X, et al. Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. Cancer Res 2002;62:7241.
34. Lang M, Henson R, Braconi C, et al. Epigallocatechin-gallate modulates chemotherapy-induced apoptosis in human cholangiocarcinoma cells. Liver Int 2009;29:670.