Metronomic Low-Dose Chemotherapy Boosts CD95-Dependent Antiangiogenic Effect of the Thrombospondin Peptide ABT-510: A Complementation Antiangiogenic Strategy

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Abstract

Blocking angiogenesis is a promising approach in cancer therapy. Natural inhibitors of angiogenesis and derivatives induce receptor-mediated signals, which often result in the endothelial cell death. Low-dose chemotherapy, given at short regular intervals with no prolonged breaks (metronomic chemotherapy), also targets angiogenesis by obliterating proliferating endothelial cells and circulating endothelial cell precursors. ABT-510, a peptide derivative of thrombospondin, kills endothelial cell by increasing CD95L, a ligand for the CD95 death receptor. However, CD95 expression itself is unaffected by ABT-510 and limits its efficacy. We found that multiple chemotherapy agents, cyclophosphamide (cytoxan), cisplatin, and docetaxel, induced endothelial CD95 in vitro and in vivo at low doses that failed to kill endothelial cells (cytoxan > cisplatin > docetaxel). Thus, we concluded that some of these agents might complement each other and together block angiogenesis with maximal efficacy. As a proof of principle, we designed an antiangiogenic cocktail combining ABT-510 with cytoxan or cisplatin. Cyclophosphamide and cisplatin synergistically increased in vivo endothelial cell apoptosis and angiogenesis by ABT-510. This synergy required CD95, as it was reversible with the CD95 decoy receptor. In a mouse model, ABT-510 and cytoxan, applied together at low doses, acted in synergy to delay tumor take, to stabilize the growth of established tumors, and to cause a long-term progression delay of PC-3 prostate carcinoma. These antitumor effects were accompanied by major decreases in microvessel density and concomitant increases of the vascular CD95, CD95L, and apoptosis. Thus, our study shows a “complementation” design of an optimal cancer treatment with the antiangiogenic peptide and a metronomic chemotherapy.

Thrombospondin-1 is a well-known antiangiogenic agent (1). Its mechanism of action and structure-function relationship have been analyzed in considerable depth, resulting in the discovery of a minimal active heptapeptide in which antiangiogenic activity is greatly enhanced by l-isoleucine to d-isoleucine replacement (2). ABT-526 and ABT-510 are modified versions of this minimal peptide with increased potencies (3) and improved clearance; ABT-510 is currently under evaluation in phase II clinical trials (4). Thrombospondin-1 has also been identified as a host-derived mediator of the antiangiogenic action of low-dose metronomic chemotherapy (5, 6). Thrombospondin-1 and ABT-510 act by inducing endothelial cell apoptosis in some cases via CD36 cell surface receptor (7, 8). Proapoptotic signal elicited by thrombospondin-1 generates CD95L, a ligand for the CD95 death receptor (9). However, CD95 expression on vascular endothelial cell is independent of thrombospondin-1; thus, accessible CD95 limits the rate of apoptosis and antiangiogenesis due to thrombospondin-1 and consequently determines, at least in part, the efficacy of thrombospondin-1-based cancer treatments.

Seeking agents to improve the efficacy of ABT-510, we turned to conventional chemotherapy drugs. Metronomic chemotherapy (low-dose chemotherapy given at close regular intervals with no prolonged drug-free breaks) provides a way to inhibit tumor angiogenesis by targeting proliferating endothelial cells in tumor blood vessels and circulating endothelial cell precursors (CEP) that are later integrated in tumor vasculature (10–12). Metronomic therapy has lower toxicity compared with pulsatile maximum tolerated dose (MTD) chemotherapy and therefore lessens or removes the need for the growth factors to accelerate recovery from myelosuppression. Moreover, despite the use of lower cumulative doses, the metronomic approach sometimes shows superior results in prolonging survival times compared with the conventional MTD regimens, in preclinical models (13, 14). Concomitant use of metronomic chemotherapy and...
antiangiogenic drugs, targeted therapies, or antitumor vaccines is an attractive combination treatment possibility as such combinations may be associated with reduced toxicity and therefore improve the quality of life (reviewed in ref. 12).

One chemotherapy agent, Adriamycin, used at low metronomic-type dosing, has been shown to increase CD95 (15) and to augment cell killing by ABT-510 in cultured endothelial cell and on remodeling vasculature in vivo (16). We aimed to determine if this "complementary" effect is restricted to Adriamycin alone, or is on the contrary, typical for multiple chemotherapy agents. We were able to show that three other compounds, cyclophosphamide (cytoxan), cisplatin, and docetaxel (Taxotere), when applied at low doses failed to induce endothelial cell or tumor cell apoptosis but increased endothelial CD95 in culture and in vivo with varying efficacy (cytoxan > cisplatin > docetaxel). We found that metronomic cytoxan and cisplatin synergistically increased angiostasis and endothelial cell apoptosis by ABT-510 in vivo. Using a monoclonal antibody and soluble Fas-Fc decoy receptor in vivo, we show that antiangiogenesis and endothelial cell apoptosis by the ABT-510/cytoxan combination relied on the CD95 death cascade.

In a mouse model of tumor therapy, ABT-510 and cytoxan also acted in synergy, delaying tumor progression, reducing tumor microvascular density, and causing endothelial cell apoptosis. Further search for the agents, which augment endothelial CD95, may yield even more potent combinations of the low-toxic metronomic chemotherapy and nontoxic ABT-510, which could result in prolonged delays in tumor progression while maintaining higher quality of life for cancer patients.

**Materials and Methods**

**Cells and reagents.** Human endothelial cells (Cambrex, Walkersville, MD) were grown in MCDB medium (Sigma, St. Louis, MO). 5% FBS.
Apoptosis was evaluated as % cells in sub-G1 phase. FACScan cytometer (BD Biosciences, Rockville, MD) using CellQuest propidium iodide and RNase and analyzed by flow cytometry on a (1 hour). The samples were incubated 45 minutes at 37°C harvested by brief trypsinization, washed, and fixed in 70% ethanol with 4-hydroperoxy-cytoxan, CP, or docetaxel in growth medium, tissue culture plates) grown to 50% confluence were treated 48 hours by trypsinization in 3 mmol/L EDTA, washed, incubated 30 minutes at 37°C with propidium iodide and RNase and analyzed by flow cytometry on a FACSscan cytometer (BD Biosciences, Rockville, MD) using CellQuest (BD Biosciences) and ModFit LT (Verity, Topsham, ME) software. Apoptosis was evaluated as % cells in sub-G1 phase.

Cell cycle analysis. The cells (1 × 10^6 per well in gelatinized six-well tissue culture plates) grown to 50% confluence were treated 48 hours with 4-hydroperoxy-cytoxan, CP, or docetaxel in growth medium, harvested by brief trypsinization, washed, and fixed in 70% ethanol (1 hour). The samples were incubated 45 minutes at 37°C with propidium iodide and RNase and analyzed by flow cytometry on a FACSscan cytometer (BD Biosciences, Rockville, MD) using CellQuest (BD Biosciences) and ModFit LT (Verity, Topsham, ME) software. Apoptosis was evaluated as % cells in sub-G1 phase.

Evaluation of CD95 expression. Similarly treated cells were harvested by trypsinization in 3 mmol/L EDTA, washed, incubated 30 minutes at 4°C with mouse anti-human CD95 or isotype control antibodies (1 μg/ml, PharMingen, San Diego, CA) followed by FITC-conjugated rabbit anti-mouse IgG (1:50; DAKO, Fort Collins, CO) and analyzed by fluorescence-activated cell sorting.

Matrigel plug angiogenesis assay. C57BL/6 mice were injected above the sternum (s.c.) with 0.4 mL Matrigel (BD Biosciences) containing 100 ng/mL vascular endothelial growth factor and 69 units/mL heparin (17) and randomized into groups of five. The treatments were 1 mg/kg/d ABT-510 or 0.1 mL vehicle saline i.p., cytoxan (2 or 20 mg/kg/d, oral), or ABT-510 + cytoxan. Tumors were measured every 2 days or weekly and the volumes calculated as \[ V = 0.5 (length \times width)^2 \]. The duration of the assay was 16 days for Lewis lung carcinoma, 35 days or 21 weeks for PC-3. In a prolonged study, ABT-510 was used at 60 mg/kg, 5 of 7 days a week and cytoxan at 20 mg/kg.

Detection of the microvessel density and apoptosis. Tumor or Matrigel sections (5 μm) were incubated with rat anti-mouse CD31 antibody (PharMingen, San Diego, CA) followed by Rhodamine-conjugated donkey anti-rat antibody (Jackson ImmunoResearch, West Grove, PA). Apoptosis was detected by in situ terminal deoxynucleotidyl transferase-mediated nick end labeling with ApopTag FITC Kit (Chemicon, Temecula, CA). Digital images were obtained by confocal microscopy (Zeiss LSM510) and quantified for microvascular density and apoptosis with ImageJ software (≥10, 40× fields per section).

Statistical and mathematical analysis. The results were evaluated using paired Student’s t-test (SigmaPlot). The difference was considered significant for \( P < 0.05 \). To evaluate the interaction between compounds fractional product of Webb was calculated using the equation \[ E_{EXP} = E_1 + E_2 - E_1 \times E_2 \] (17). For limited isobolar analysis.

Fig. 2. Low-dose chemotherapy augmented endothelial cell death due to ABT-510 via CD95-dependent mechanism. A, mice were given s.c. Matrigel plugs and treated with indicated compounds (CTX-cytoxan, CDDP-cisplatin, grid). The plugs were sectioned, endothelial structures visualized by CD31 fluorescent staining (red) and apoptosis detected by terminal deoxynucleotidyl transferase–mediated nick end labeling (green). Note increase in apoptotic endothelial cells by combined treatments. B, low-dose chemotherapy augmented angiogenesis suppression by ABT-510. Mice bearing angiogenic Matrigel plugs were treated as indicated. At the end-point, the plugs were processed in (A) and microvascular density (MVD) quantified using LasePfx software (Bio-Rad). C-D, angiogenesis reduction was due to CD95-dependent apoptosis mice bearing 1Matrigel plugs supplemented with vascular endothelial growth factor and heparin were treated with the combination of ABT-510 and cytoxan (CTX; 1 mg/kg i.p. and 2 mg/kg orally, respectively), alone or in combination with Fas-Fc decoy receptor, a neutralizing antibody against CD36, an ABT-510 receptor, or an isotype control IgA antibody. The plugs were processed as in (A); angiogenesis (C) and apoptosis (D) quantified as above. Note the reversal of antiangiogenesis and apoptosis by CD36 antibody or by Fas decoy.

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(18), the interaction index was calculated as: $I = \sum \alpha_i^2 (i = 1, 2, 3 \ldots)$, where $d_i$ is the dose of a compound in combined treatment and $D_i$ is the dose producing the same effect as a solitary agent. In case of synergy, we expect $E_{\text{Exp}} < E_{\text{Obs}}$ (the observed effect of combined treatment) and $I < 1.0$. Finally, the equation by Chou and Talalay (19), $D = D_m \times (E/E_1 - E)^{1/\alpha}$, was used to determine the expected concentration for the individual compounds, which were subsequently used to calculate the fractional product of Webb.

Results

**Multiple chemotherapy agents at low-dose induced endothelial CD95 in vitro.** Seeking therapies capable of synergy with ABT-510, we have tested three chemotherapy agents distinct in their mechanisms of killing cancer cells: cytoxan, an alkylating agent, a DNA-damaging agent, cisplatin, and docetaxel, which causes microtubule stabilization and thus leads to mitotic arrest and apoptosis. Surprisingly, all three compounds induced CD95 presentation by quiescent endothelial cell in vitro albeit with varying efficacy (Fig. 1A). Cytoxan effectively induced CD95 at $\geq 5$ nmol/L. On the other hand, endothelial cell apoptosis by cytoxan became detectable only at doses above 10 nmol/L. Interestingly, cytoxan simultaneously induced CD95 presentation and apoptosis (up to 60%) in the PC-3 prostate cancer cells at $\sim 12$ nmol/L (data not shown). Cisplatin significantly increased CD95 on endothelial cell but not on PC-3 cells at 4 to 6 nmol/L. At this dose of cisplatin, endothelial cell apoptosis remained similar to untreated control (<3%). Docetaxel caused a modest CD95 increase in quiescent endothelial cells but only at doses where apoptosis already became prominent.

Both cytoxan and cisplatin enhanced CD95 presentation by remodeling microvascuature in vivo. In mice bearing s.c. Matrigel plugs containing vascular endothelial growth factor/ heparin mix to induce angiogenesis, daily treatments with 2 mg/kg cytoxan or with 0.4 mg/kg cisplatin every 3 days significantly increased the number of CD95-positive endothelial structures (capillaries) detected by in situ immunofluorescence (Fig. 1B). Quantitative analysis revealed statistically significant increases in the endothelial CD95 in mice treated with cisplatin and cytoxan (4- to 6-fold, $P < 0.0003$; Fig. 1C). As was expected from the in vitro observations, docetaxel caused only a mild, albeit statistically significant 1.5-fold CD95 induction in vivo ($P < 0.05$; data not shown).

**Low-dose chemotherapy enhanced ABT-510 activity by augmenting CD95-dependent endothelial cell apoptosis.** We then tested cytoxan and cisplatin at low-metronomic doses for the ability to block angiogenesis and to induce endothelial cell apoptosis alone or in combination with antiangiogenic ABT-510. Because ABT-510 causes endothelial cell death via a CD95-dependent mechanism, we expected it to cooperate with the agents that enhance CD95 presentation. Indeed, both 4-hydroperoxy-cytoxan and cisplatin enhanced endothelial cell apoptosis by ABT-510 in vitro (Fig. 1D; data not shown). At 1 nmol/L 4-hydroperoxy-cytoxan and cisplatin altered neither endothelial cell CD95 presentation nor ED$_{50}$ of ABT-510 (~30 nmol/L). However, in the presence of 5 nmol/L 4-hydroperoxy-cytoxan or cisplatin, ABT-510 ED$_{50}$ value dropped to ~1 and ~3 nmol/L, respectively. To measure microvascular density and endothelial cell apoptosis in vivo, we used Matrigel plugs implanted in mice treated with ABT-510 and/or cytoxan or cisplatin (Fig. 2). Visual evaluation suggested a weak microvascular density reduction and low-level apoptosis by ABT-510 at a chosen low dose and by metronomic-type dosing of cytoxan and cisplatin in the Matrigel model of angiogenesis. However, combining these treatments caused a substantial increase in the endothelial cell apoptosis and a dramatic microvascular density reduction (Fig. 2A). Quantitative analysis showed a trend towards decreased microvascular density by cytoxan at 2 or 20 mg/kg/d in a 7-day assay, which failed to reach statistical significance ($P > 0.36$). ABT-510 at 1 mg/kg caused a mild albeit significant microvascular density reduction (1.2-fold, $P \leq 0.05$), which was greatly augmented by cytoxan at 2 and 20 mg/kg (3.7- and 2.3-fold, respectively; $P < 0.0004$). Cisplatin by itself, at 4 mg/kg reduced microvascular density by the factor of 1.7 ($P < 0.01$), whereas combined with ABT-510, it caused a more substantial 2.8-fold decrease in microvascular density ($P \leq 0.05$). Limited isobologram analysis showed synergistic antiangiogenic activity between ABT-510 and cytoxan at 2 mg/kg, whereas 20 mg/kg cytoxan and 0.4 mg/kg cisplatin did not exceed additive.

Synergistic antiangiogenic effect of ABT-510 and low-dose cytoxan was concomitant with apoptosis induction (Fig. 2C-D). Both apoptosis and antiangiogenesis by cytoxan/ABT-510 combination were critically dependent on CD95 signaling. When soluble Fas-Fc decoy receptor was incorporated in the Matrigel plugs, it completely abolished the reduction of microvascular density by the ABT-510/cytoxan combination and blocked endothelial cell apoptosis in vivo (Fig. 2C-D). Neutralizing antibody against CD36, a thrombospondin-1 antiangiogenic receptor, also abolished the effect of ABT-510 and of the ABT/cytoxan combination, pointing to a requirement for the CD36-dependent increase in CD95L (Fig. 2C-D).

In vitro CD95 expression and ABT-510-driven endothelial cell apoptosis were directly proportional to cytoxan concentration (Fig. 1A and D). Thus, the less-pronounced endothelial cell apoptosis in response to a higher cytoxan dosing was surprising. However, 4-hydroperoxy-cytoxan at doses of >10 nmol/L directly induced endothelial cell apoptosis with faster kinetics than ABT-510.® Earlier onset of apoptosis in vivo due to the higher cytoxan dose may be followed by endothelial cell autophagy, which could hamper our ability for detection.

**ABT-510 and metronomic cytoxan cooperatively blocked tumor growth.** We then tested the ability of CD95-inducing agents to cooperate with ABT-510 in blocking tumor growth using syngeneic mouse model and xenograft tumors in immune deficient mice. Because cytoxan and ABT-510 showed synergy, in contrast with cisplatin whose effect was merely additive, we focused on the cytoxan/ABT-510 combination. The growth of syngeneic Lewis lung carcinoma was unaffected by the solitary ABT-510 at 1 mg/kg or by cytoxan at 2 mg/kg. Combined, ABT-510 and cytoxan caused a weak albeit significant decrease in the Lewis lung carcinoma tumor volume (Fig. 3A; $P \leq 0.05$). Similar growth reduction could be achieved with an extremely high dose of ABT-510 alone (240 mg/kg bid; data not shown). The growth of PC-3 prostate carcinoma grafted to the immune deficient mice (nu/nu) was moderately delayed (~30%) by both ABT-510 and cytoxan at 1 and 20 mg/kg, respectively, whereas combined therapy caused a significant delay of $\geq 60\%$ (Fig. 3B; $P \leq 0.04$). Combined treatment with the low-dose ABT-510 and metronomic cytoxan caused stabilization of...

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5 R. Yap and O.V. Volpert, unpublished data.
established tumors. If identical treatments were initiated when the tumors reached a minimal volume of 250 mm$^3$, those in the groups treated with solitary compounds grew roughly at the rates similar to those of control group. In contrast, the tumors in the group treated with ABT-510/cytoxan combination remained stable (~75% smaller than in control group; Fig. 3C; $P \leq 0.02$).

To clearly show synergy between ABT-510 and cytoxan, we used a short-term tumor study with suboptimal doses of both antiangiogenic ABT-510 and of metronomic cytoxan chemotherapy (see above), which are minimally effective as solitary compounds. Subsequently, to determine the effect of this synergistic combination on the long-term growth of PC-3 xenograft tumors, we used the doses determined as maximally effective in previous studies (6, 8, 10), ABT-510 at 60 mg/kg/d and cytoxan at 20 mg/kg/d (Fig. 3D). The treatment was commenced on day 29, after the tumors became palpable (100-150 mm$^3$). Solitary ABT-510 caused the delay in tumor growth that was barely significant ($P < 0.04$ by treatment week 7). Cytoxan at 20 mg/kg produced a substantial 7-week progression delay compared with the sham-treated control and a 6-week delay compared with ABT-510 ($P \leq 0.02$). Importantly, combined ABT-510 and cytoxan produced an effect far exceeding additive and extended progression delays to at least 11 weeks. Moreover, whereas the reduction in tumor size in a group receiving solitary ABT-510 was insignificant ($P = 0.76$), and cytoxan alone reduced tumor volume by 75% to 80% ($P \leq 0.001$), adding ABT-510 to cytoxan metronomic treatment reduced tumor volume by further 2.8-fold ($P \leq 0.03$) causing a total 12.6-fold decrease.

During the first 4 weeks of treatment, the mice treated with metronomic cytoxan, ABT-510, and ABT-510 + cytoxan continued to gain weight similarly to the control group. After day 28, animals treated with regimens containing cytoxan showed a slightly slower weight gain than the groups treated with ABT-510 or vehicle control, likely due to the cytoxan arm of the treatment. No overt weight loss was noted during long-term treatment period suggesting mild or no toxic effects (data not shown).

ABT-510/cytoxan combination synergistically increased apoptosis in the tumor-associated endothelium and obliterated microvasculature. We compared microvascular density and the endothelial cell apoptosis between distinct treatment groups (Fig. 4A-C). Quantitative analysis of the data is summarized in Table 1. In all tumor models, both cytoxan and ABT-510 induced significant amount of apoptosis and reduced tumor microvascular density. However, cumulative action of the cytoxan/ABT combination far exceeded simple additive effect, as was determined using fractional product of Webb calculations (18). Low doses of ABT-510 and of cytoxan were
Fig. 4. Combined treatment with anti-angiogenic ABT-510 and metronomic cytoxan (CTX) worked in synergy to block angiogenesis and to induce apoptosis. Tumors from experiments in Fig. 3 were frozen, sectioned, stained for CD31, apoptosis detected by *in situ* terminal deoxynucleotidyl transferase–mediated nick end labeling, and confocal images obtained and quantified using LaserPix software. A, microvascular density (MVD, left) and apoptosis (right) in Lewis lung carcinoma (LLC) flank tumors. Treatment indicated below. B, microvascular density (left) and apoptosis (right) in PC-3 tumors treated with low doses of ABT-510 and cytoxan. C, microvessel density (left) and apoptosis (right) in PC-3 tumors treated with higher doses of ABT-510 and metronomic cytoxan. D, decreased microvessel density and apoptosis correlated with increased expression of CD95 and CD95L. Cryosections of tumors from mice treated as indicated (left) were stained for CD31 (red), CD95 (blue), and CD95L (green). Note higher CD95 in cytoxan-treated tumors and increased CD95L in tumors treated with ABT-510.
Discussion

The concept of low-dose metronomic chemotherapy as an antiangiogenic treatment developed quickly from preclinical models into clinical trial evaluation (12). It offers a promising alternative to more toxic conventional MTD chemotherapy regimens. Alternatively, brief initial treatment with MTD regimens followed by long-term metronomic regimens holds great promise when dealing with bulky tumors, which show major response to MTD therapy (20). Adding targeted anticancer drugs, especially angiogenesis inhibitors, to metronomic chemotherapy for chronic combination treatment is another promising means of development (12, 20, 21).

A number of preclinical studies have implicated angiogenesis blockade in the antitumor effects of metronomic chemotherapy (12, 22), where targeting of the growing neovasculature led to a secondary antitumor effect. There are three known nonmutually exclusive mechanisms underlying antiangiogenic effect of metronomic chemotherapy (12). The first is by direct induction of apoptosis of presumably proliferating tumor endothelium (21, 22), the second is by blocking the mobilization or reducing the numbers of viable circulating endothelial progenitor cells which contribute to tumor neovascularization (10, 11). The third mechanism operates by elevating the levels of the cellular and circulating angiogenesis inhibitor, thrombospondin-1 via unknown steps (5, 6). The increase in thrombospondin-1 and not direct killing by the cytotoxic chemotherapy drugs may be also responsible for the apoptosis of fully differentiated endothelial cells due to metronomic chemotherapy as well as the suppression of CEPs. Indeed, thrombospondin-1 peptide mimetic ABT-510 suppressed CEP levels even in drug-treated mice (10). Thrombospondin-1 also lowers the numbers of the CEPs, whose incorporation in the remodeling vessels provides a necessary component of tumor angiogenesis (6, 10). Thus, the development of strategies aimed to enhance apoptosis of the endothelial cells (and possibly CEP) by metronomic chemotherapy, especially those, which exploit thrombospondin-1 assume a high priority.

We devised a “complementation” strategy in which the ability of thrombospondin-1 to target tumor is enhanced by additional sensitization of endothelial cells. In this specific approach antiangiogenic peptide, ABT-510 was complemented by a metronomic chemotherapy regimen that increases CD95, the rate-limiting intermediate of the parental thrombospondin-1 whose effect is mimicked by ABT-510 (23). In addition to Adriamycin, we identified two more agents, cytoxan and cisplatinum, that increased endothelial cell associated CD95 and significantly augmented apoptotic, antiangiogenic, and antitumor effects by the ABT-510. In a short-term assay, the combination of ABT-510 and metronomic cytoxan showed definite synergy in delaying tumor progression and stabilizing the growth of established tumors. In a prolonged assay, higher dose of the ABT-510 had only modest effect but was strongly synergistic with the metronomic cytoxan treatment. This synergistic antitumor effect was associated with decreased angiogenesis and increased apoptosis of the tumor endothelium, which coincided with concomitantly elevated levels of proapoptotic CD95 and CD95L. Our immediate goal was to establish the proof of principle for the complementation therapies and to provide starting material for further analysis and optimization of combination treatments. The delay in tumor take can be viewed as a model for testing treatments aimed to control dormant metastases, for which chronic treatment would be desirable. Because in a chronic treatment toxic effects accumulate over time, we used minimal effective doses of both antiangiogenic and chemotherapy agent. In a more acute model of established tumor, we tested both lower and higher cytoxan doses.

It is important to note that thrombospondin-1 is not the only agent that uses CD95 cascade in killing activated endothelium. Pigment epithelial-derived factor, a potent antiangiogenic protein also up-regulates CD95L as a part of antiangiogenic signal (9). Recently, two other natural inhibitors, Canstatin and Angiostatin, have also been shown to rely, at least in part, on moderately synergistic, whereas optimal, higher displayed strong synergy in PC-3 xenograft models ($E_{\text{obs}} > 2 \times E_{\text{exp}}$; Table 1; $P < 0.01$). Interestingly, increased apoptosis and a stronger decrease in microvascular density coincided with concomitant expression of CD95/CD95L by the tumor vasculature (Fig. 4D).

<table>
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<th>Tumor model</th>
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CD95-mediated apoptosis (24, 25). It may be that the reason for success in combining antiangiogenic agents such as thrombospondin-1, angiostatin, or a noncatalytic fragment of matrix metalloproteinase 2 termed PEX with metronomic carboplatin or etoposide (26) lies in the elevated CD95 expression levels induced by chemotherapeutic agents, which complement the angioinhibitory molecular events.

There are other possible added benefits in combining if antiangiogenic drugs with the metronomic chemotherapy. For example, one of them, reduced oncotic pressure due to normalization of the tumor vasculature, improves the delivery of chemotherapeutic drugs to the tumor cells thus diminishing at least one possible component of intrinsic tumor resistance to chemotherapy (27). On the other hand, CD95 expression by tumor cells noted in our study may contribute to the tumor cell killing by “fratricide” due to ABT-510-dependent expression of CD95L by the tumor vasculature.

The cumulative doses during metronomic chemotherapy is significantly lower than in corresponding MTD regimen and likewise are the associated toxic effects (28). Similarly, toxicity associated with the use of the natural (endogenous) angiogenesis inhibitors observed during clinical trials was noted as very modest (4). Therefore, combination therapies based on the use of naturally occurring angiogenesis inhibitors and metronomic chemotherapy hold considerable promise not only as and efficacious treatment but especially as a relatively nontoxic anticancer strategy thus helping to improve the quality of life of cancer patients receiving chemotherapy agents.

References

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