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Paclitaxel/carboplatin administration along with antiangiogenic therapy in non-small-cell lung and breast carcinoma models

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Abstract *Introduction*: With the introduction of new drugs, the efficacy of chemotherapy in non-small-cell lung cancer has been improving. The combination of paclitaxel and carboplatin has shown activity in this disease but is far from curative. *Methods*: The antiangiogenic agent regimen of TNP-470/minocycline was added to treatment with paclitaxel and carboplatin alone and in combination in animals bearing the Lewis lung carcinoma. *Results*: Administration of the antiangiogenic regimen prior to, during and after the cytotoxic therapy increased the tumor growth delay 1.6-fold and decreased the number of lung metastases to 20% of the number observed in the control animals. [^{14}C]Paclitaxel, platinum from carboplatin and [^{14}C]albumin levels were determined over a 24-h time course in tumors and normal tissues of animals bearing the Lewis lung carcinoma and pretreated with TNP-470/minocycline or not pretreated. There were higher levels of [^{14}C]paclitaxel, platinum from carboplatin and [^{14}C]albumin in the tumors and some normal tissues of the animals that had received TNP-470/minocycline compared with those that had not received TNP-470/minocycline, especially at the earlier time points. Administration of TNP-470/minocycline to animals bearing the EMT-6 mammary carcinoma increased the cytotoxicity of high-dose paclitaxel toward EMT-6 tumor cells and toward bone marrow CFU-GM. Administration of TNP-470/minocycline to animals bearing the EMT-6 mammary carcinoma also increased the cytotoxicity of carboplatin toward the EMT-6 tumor cells but did not affect the toxicity of carboplatin toward the bone marrow CFU-GM. *Conclusions*: The addition of TNP-470/minocycline to treatment with paclitaxel and carboplatin resulted in increased antitumor activity and efficacy and further investigation of this combination is warranted.

Key words TNP-470 · Antiangiogenic agents · TNP-470/minocycline

Introduction

Given the relatively modest impact of established combination chemotherapy regimens on survival in advanced non-small-cell lung cancer, the development of new treatments for this very common malignancy is imperative. Among the newer chemotherapeutic agents, the taxane paclitaxel has demonstrated significant activity against metastatic non-small-cell lung cancer as a single agent with much improved median survival [6, 18]. Since platinum-based therapeutic combinations have been historically important in the treatment of non-small-cell lung cancer, several phase II studies have been conducted combining administration of paclitaxel and carboplatin [11, 16, 17, 20, 35]. These phase II studies have produced promising results showing that the combination of paclitaxel and carboplatin is an active and generally well-tolerated regimen for non-small-cell lung cancer. This two-drug regimen produces response rates between 30% and 50% and prolonged median survival of >1 year. Paclitaxel/carboplatin is not curative in advanced non-small-cell lung cancer and complete responses are rare.

Potentiation of the efficacy of cytotoxic anticancer therapies by administration of antiangiogenic agents has been demonstrated in several in vivo experimental systems [1, 27–31, 33]. Among the most promising of the antiangiogenic agents is TNP-470, a synthetic analog of the fungal antibiotic fumagillin. TNP-470 is a potent inhibitor of endothelial cell migration [4], endothelial

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cell proliferation [2, 10, 15, 36] and capillary tube formation [14]. TNP-470 also inhibits angiogenesis as demonstrated in the chick chorioallantoic membrane (CAM) assay, and the rabbit and the rodent cornea assays [14]. TNP-470 has been shown to inhibit the growth of certain primary and metastatic murine tumors as well as human tumor xenografts [3, 5, 13, 22, 34, 37–39]. TNP-470 undergoes a rapid metabolism in vivo [19].

The current study was undertaken to assess the efficacy of TNP-470/minocycline in a treatment regimen including paclitaxel and carboplatin.

Materials and methods

Drugs

TNP-470 (AGM-1470) was a gift from Dr. Deborah Milkowski, TAP Pharmaceuticals (Deerfield, Ill). Carboplatin and paclitaxel, and [^{14}C]paclitaxel (2-benzoyl-ring-UL-^{14}C; 40–60 mCi/mmol) and [^{14}C]albumin (^{14}C-methylated bovine albumin; 40 μCi/mg protein) were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Tumor

The Lewis lung tumor [7, 8, 21] was carried in male C57BL mice (Taconic Laboratories, Germantown, N.Y.). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted subcutaneously into the legs of male mice aged 8–10 weeks. Tumors reach a volume of 100 mm^3 by day 7 after tumor cell implantation.

Tissue distribution

Animals bearing Lewis lung tumors were treated on alternate days by subcutaneous injection of TNP-470 (30 mg/kg) days 4–8 and daily by intraperitoneal injection of minocycline (10 mg/kg) on days 4–8 following tumor implantation. On day 8 these pretreated animals and other tumor-bearing animals that were not pretreated were injected intravenously with 3.1 μCi of [^{14}C]paclitaxel (36 mg/kg), 0.65 μCi of [^{14}C]albumin (10 mg/kg) or carboplatin (300 mg/kg). At 1 min, 15 min, 30 min, 1 h, 3 h, 6 h, 15 h and 24 h after administration of each agent two animals were killed. Known wet weights of tumor, liver, kidney, brain, heart, gut, lung, skin, skeletal muscle, and blood from the animals injected with [^{14}C]paclitaxel or [^{14}C]albumin were dissolved in a tissue solubilizer (Protosol; DuPont Biotechnology Systems), then counted by liquid scintillation in Aquasol (DuPont Biotechnology Systems) [26]. Known wet weights of the same tissues from the animals injected with carboplatin were dissolved in the protosol tissue solubilizer, then analyzed by flameless atomic absorption spectroscopy. Platinum from a 15-μl sample injection volume was atomized from the walls of pyrolytically coated graphite tubes. A Perkin-Elmer Model 2380 atomic absorption spectrophotometer was used in conjunction with a Perkin-Elmer Model 400 graphite furnace to measure the absolute mass of platinum in the cell samples. Each measurement was made in triplicate [9, 26].

Tumor cell-survival assay

The EMT-6/Parent mouse mammary carcinoma grown as a solid tumor subcutaneously in the flanks of female Balb/C mice (Taconic Farms, Germantown, N.Y.) has been used widely in radiobiology and chemotherapy studies. The EMT-6 murine mammary carcinoma is an in vivo–in vitro tumor system [26]. The EMT-6/Parent and alkylating agent-resistant tumors were grown in female Balb/C mice. For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted subcutaneously into the hind legs of Balb/C mice aged 8–10 weeks. Tumor cell survival was performed when the tumors had reached a volume of approximately 150 mm^3 (day 9 after tumor implantation). On day 8 the animals were treated with single doses of paclitaxel (24, 36 or 48 mg/kg) by intravenous injection or with carboplatin (100, 300 or 500 mg/kg) by intraperitoneal injection alone or following pretreatment with TNP-470 (30 mg/kg) administered by subcutaneous injection and minocycline (10 mg/kg) administered by intraperitoneal injection on days 4 through 8.

A 24-h interval was incorporated before the mice were killed to allow for the full expression of drug cytotoxicity and repair of potentially lethal damage. Mice were immersed briefly in 95% ethanol and the tumors were excised under sterile conditions in a laminar flow hood and minced to a fine brei with two scalpels. Four tumors were pooled to make each treatment group. Approximately 400 mg tumor brei was used to make each single-cell suspension. All reagents were sterilized with 0.22 μm Millipore filters and were added aseptically to the tumor cells.

Each sample was washed in 20 ml Waymouth's medium (Mediatech, Pittsburgh, Pa.) after which the liquid was gently decanted and discarded. The samples were resuspended in 450 U collagenase/ml (Sigma, St. Louis, Mo.) and 0.1 DNase/ml (Sigma) and incubated for 10 min at 37 °C in a shaking water bath. The samples were resuspended as described above and incubated for another 15 min at 37 °C. Next, 1 ml of 1 mg/ml DNase was added and incubation was continued for 5 min at 37 °C. The samples were then filtered through a 70-μm cell strainer (Fisher, Pittsburgh, Pa.). The samples were washed twice, then resuspended in Waymouth's medium supplemented with 15% newborn calf serum. These single-cell suspensions were counted and plated at six different cell concentrations for the colony-forming assay. No significant difference was observed in the total cell yield from the pooled tumors in any treatment group. After 1 week, the plates were stained with crystal violet and colonies of more than 50 cells were counted. The untreated tumor cell suspensions had a plating efficiency of 8–14%. The results are expressed as the surviving fraction (±SE) of cells from the untreated groups as compared with untreated controls.

Bone marrow toxicity

Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle and the granulocyte-macrophage colony-forming unit (CFU-GM) assay was carried out as described previously [25]. Bone marrow cells were suspended in supplemented McCoy's 5A medium containing 15% fetal bovine serum (FBS), 0.3% agar (Difco, Detroit, Mich.), and 10% conditioned medium as a source of colony-stimulating activity. The colony-stimulating activity supplement was prepared by incubating L-929 mouse fibroblasts (2500 cells/ml; Microbiological Associates, Bethesda, Md.) with 30% FBS in McCoy's 5A medium for 7 days at 37 °C in a humidified atmosphere containing 5% CO_2. The supernatant containing the colony-stimulating activity was obtained by centrifugation of the medium at 10 000 *g* for 10 min at 4 °C followed by filtration under sterile conditions. The bone marrow cell cultures were incubated for 7 days at 37 °C and were fixed with 10% glutaraldehyde. Colonies of at least 50 cells were scored. The results of three experiments, in which each group was measured at six cell concentrations, were averaged. The results are expressed as the surviving fraction (±SE) of treated groups as compared with untreated controls.

Tumor growth delay experiments

By day 4 after tumor-cell implantation, Lewis lung tumors had begun neovascularization. Animals bearing Lewis lung tumors were injected subcutaneously with TNP-470 (30 mg/kg) on

Table 1 Growth delay of the Lewis lung carcinoma and number of lung metastases on day 20 after treatment of the animals with paclitaxel and/or carboplatin with or without antiangiogenic agents. Values are means ± SE of 15 animals

Treatment group	Tumor growth delay [a] (days)	Number of lung metastases
Controls	–	40 ± 7
TNP-470 (30 mg/kg) s.c., alternate days 4–18 + minocycline (10 mg/kg) i.p., days 4–18	1.0 ± 0.3	27 ± 5$^{\#}$
Paclitaxel (36 mg/kg) i.v. days 7–11	4.6 ± 0.3	22 ± 4$^{\#}$
TNP/minocycline/paclitaxel	6.4 ± 0.4*	20 ± 4$^{\#}$
Carboplatin (50 mg/kg) i.p., day 7	4.2 ± 0.3	25 ± 4$^{\#}$
TNP/minocycline/carboplatin	7.8 ± 0.5**	21 ± 3$^{\#}$
Paclitaxel/carboplatin	6.6 ± 0.4	13 ± 2$^{\#\#}$
TNP/minocycline/paclitaxel/carboplatin	10.5 ± 0.6*	8 ± 1$^{\#\#}$

* $P < 0.01$, ** $P < 0.005$, vs cytotoxic therapy alone
$^{\#}$ $P < 0.01$, $^{\#\#}$ $P < 0.005$, vs controls
[a] Tumor growth delay is the difference in days for treated tumors to reach 500 mm^3 compared with untreated control tumors. Untreated control tumors reach 500 mm^3 in about 12.4 ± 0.3 days

alternate days for eight injections, beginning on day 4, and/or were treated with minocycline (10 mg/kg) by intraperitoneal injection daily on days 4 through 18 after tumor implantation. When the Lewis lung tumors were approximately 100 mm^3 in volume on day 7 after tumor cell implantation, cytotoxic therapy was initiated. Paclitaxel (36 mg/kg) was administered by intravenous injection on days 7 through 11. Carboplatin (50 mg/kg) was administered by intraperitoneal injection on day 7.

The progress of each tumor was measured thrice weekly until it reached a volume of 500 mm^3. Tumor growth delay was calculated as the days taken by each individual tumor to reach 500 mm^3 as compared with the untreated controls. Each treatment group consisted of five animals, and the experiment was repeated three times. Days of tumor growth delay are the mean ± SE for the treatment group compared with the control [29].

Lung metastases

The external lung metastases from animals treated as described above on day 20 after tumor implantation were counted manually and scored as ≥3 mm in diameter. The data shown are the means from 6 to 12 pairs of lungs. Untreated control animals died from lung metastases on days 21 to 25 [29].

Results

The Lewis lung carcinoma growing in C57BL mice was chosen for these studies because it is a syngeneic model of non-small-cell lung cancer, it is relatively resistant to many cancer therapies and it metastasizes avidly to the lungs of the animals from subcutaneously implanted primary tumors. Treatment of Lewis lung carcinoma-bearing mice with TNP-470 (30 mg/kg) administered subcutaneously on alternate days for eight injections beginning on day 4 after tumor cell implantation and with minocycline (10 mg/kg) administered intraperitoneally daily for 15 injections beginning on day 4 after tumor cell implantation did not alter the tumor growth compared with untreated tumors (Table 1). Paclitaxel (36 mg/kg) administered by intravenous injection on days 7 through 11 after tumor cell implantation produced 4.6 days of tumor growth delay which was increased 1.4-fold to 6.4 days of tumor growth delay when administered along with TNP-470 and minocycline. A single intraperitoneal injection of carboplatin (50 mg/kg) on day 7 after tumor cell implantation produced a tumor growth delay of 4.2 days. When carboplatin was administered along with TNP-470 and minocycline a tumor growth delay of 7.8 days resulted, a 1.9-fold increase compared with carboplatin alone. The combination of the cytotoxic anticancer drugs, paclitaxel and carboplatin, was well tolerated by the animals and produced a tumor growth delay of 6.6 days. The complete regimen including TNP-470 and minocycline along with paclitaxel and carboplatin produced a tumor growth delay of 10.5 days, a 1.6-fold increase compared with the cytotoxic drug combination alone.

Treatment with the antiangiogenic agent combination decreased the number of lung metastases on day 20 to 68% of the number found in untreated control animals (Table 1). Both of the cytotoxic chemotherapeutic agents also decreased the number of lung metastases on day 20. Paclitaxel administration decreased the number of lung metastases to 55% of the number in the untreated control animals, which was not significantly altered by the coadministration of TNP-470/minocycline. Treatment with carboplatin decreased the number of lung metastases to 63% of the number in the control animals. Addition of TNP-470/minocycline administration to treatment with carboplatin did not significantly alter the number of lung metastases compared with carboplatin alone. The combination of the cytotoxic drugs reduced the number of lung metastases to 33% of the number in the control animals. With the addition of TNP-470/minocycline to the combination of cytotoxic anticancer drugs the number of lung metastases was reduced to 20% of the number in the control animals.

[^{14}C]Paclitaxel was administered to Lewis lung carcinoma-bearing animals pretreated with TNP-470/minocycline or not pretreated on day 8 after tumor implantation and tissues were collected over a 24-h time course (Fig. 1). At early time points (1 min and 15 min) after intravenous administration of the [^{14}C]paclitaxel there was a fivefold higher concentration of the drug in the tumors of animals that had been pretreated with TNP-470/minocycline. At the intermediate time points the [^{14}C]paclitaxel levels were similar in both the pretreated animals and those that had not been treated with

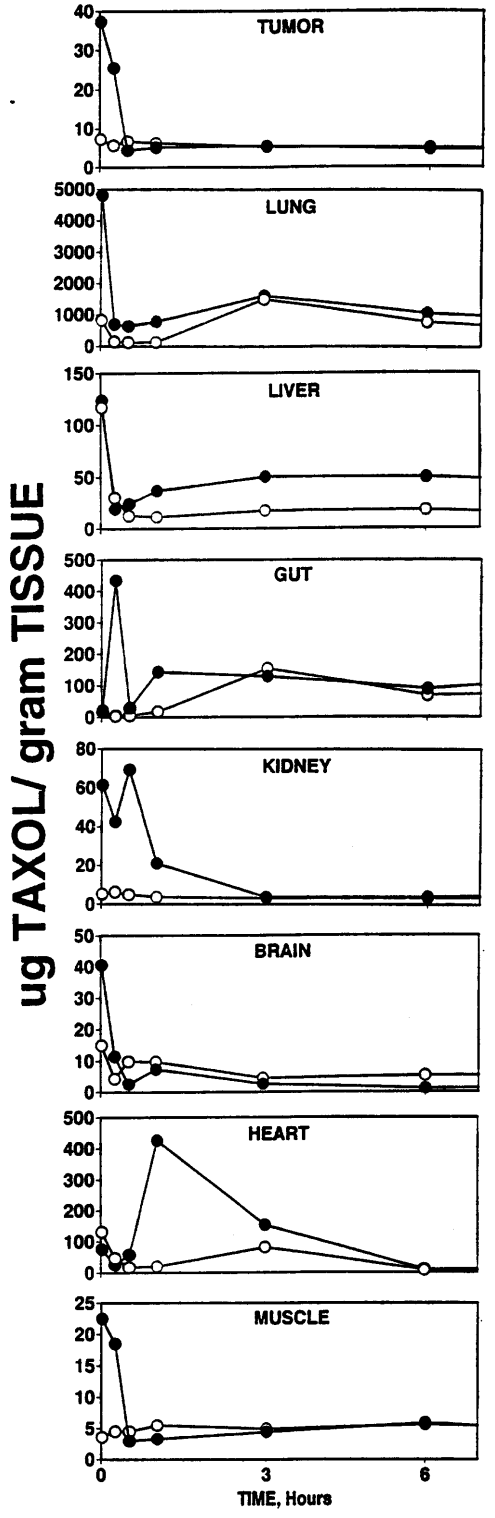

Fig. 1 Tissue levels of ^{14}C from [^{14}C]paclitaxel in C57BL mice bearing Lewis lung tumors subcutaneously in the hind leg over time after intravenous injection of 36 mg/kg of the drug alone on day 8 (○) or after administration of the drug to animals treated with TNP-470 (30 mg/kg, s.c.) days 4, 6 and 8 and minocycline (10 mg/kg, i.p.) daily days 4–8 after tumor cell implantation (●)

TNP-470/minocycline; however, by 24 h there was a twofold greater concentration of [^{14}C]paclitaxel in the tumors of the animals pretreated with TNP-470/minocycline compared with those that had not received the antiangiogenic therapy. The pattern of [^{14}C]paclitaxel distribution into the other tissues was similar with greater peak levels of [^{14}C]paclitaxel in the tissues of animals pretreated with TNP-470/minocycline. In the liver, however, there was a prolonged increased level of [^{14}C]paclitaxel of the pretreated animals. By far the highest levels of [^{14}C]paclitaxel were found in the lungs of the animals where the peak level in the pretreated animals reached 4800 μg/g tissue. Other tissues with relatively high paclitaxel concentrations were gut and heart.

Concentrations of platinum from carboplatin were two- and threefold higher in the tumors of animals pretreated with TNP-470/minocycline at 15 min and 30 min after drug administration than in the tumors of animals that had not received the antiangiogenic therapy, respectively (Fig. 2). Between 6 h and 24 h after carboplatin administration, platinum levels in the tumors of pretreated animals remained about twofold higher than in animals that did not receive the antiangiogenic therapy. Overall the tissues of the animals pretreated with TNP-470/minocycline had higher platinum levels with the greatest differentials being in kidney, brain, muscle and liver. The highest platinum levels overall were in kidney, gut, liver and brain.

To determine whether pretreatment with TNP-470/minocycline might also alter the tissue distribution of large molecules into tumors and tissues, [^{14}C]albumin was administered to TNP-470/minocycline pretreated and non-pretreated animals (Fig. 3). There was a two- to threefold higher concentration of [^{14}C]albumin in the tumor over the first hour after protein injection and a concentration differential with higher concentrations in the tumors of the pretreated animals persisting over the 24 h examined. A similar pattern pertained for the other tissues, with TNP-470/minocycline-pretreated animals having higher tissue concentrations of [^{14}C]albumin than the tissues of non-pretreated animals. The highest peak levels of [^{14}C]albumin were in liver and lung.

In animals bearing the EMT-6 mammary carcinoma, treatment with TNP-470/minocycline for 4 days prior to and at the time of a single dose of paclitaxel increased the tumor cell killing by the drug only at the highest dose of paclitaxel tested where there was a fivefold increase in tumor cell killing in animals receiving TNP-470/minocycline (Fig. 4). The survival of bone marrow CFU-GM was used as a representative sensitive normal tissue. At the highest dose of paclitaxel tested, there was a twofold diminution in the killing of bone marrow CFU-GM when the animals received TNP-470/minocycline. Therefore, the therapeutic index as determined by the ratio of tumor cell killing to bone marrow CFU-GM killing of paclitaxel was improved by the administration of TNP-470/minocycline.

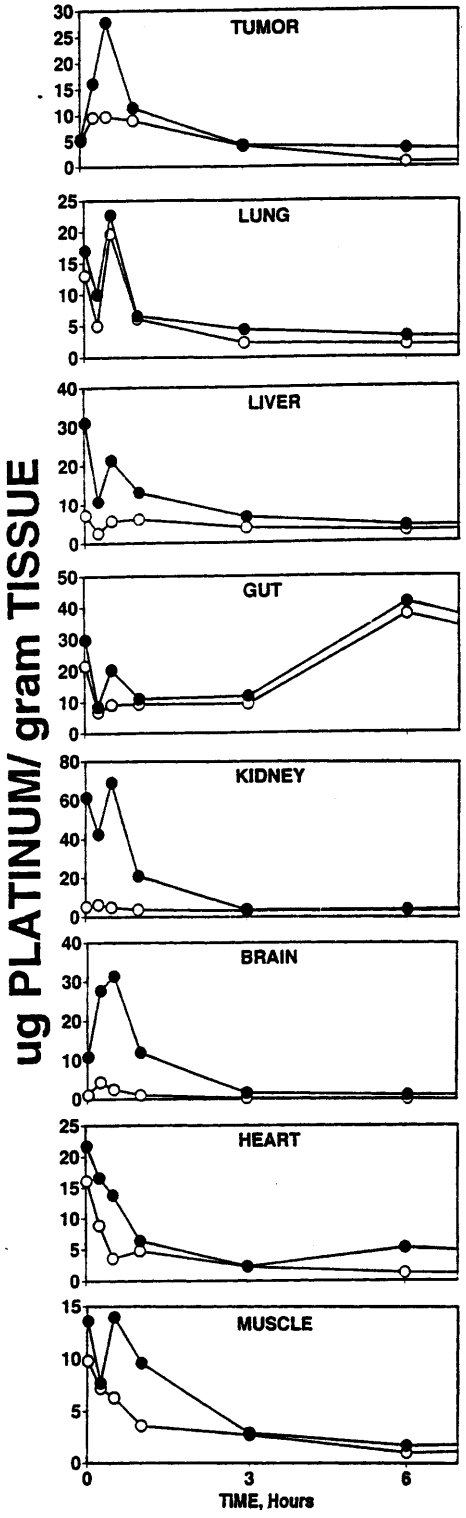

Fig. 2 Tissue levels of platinum from carboplatin in C57BL mice bearing Lewis lung tumors subcutaneously in the hind leg over time after intravenous injection of 300 mg/kg of the drug alone on day 8 (○) or after administration of TNP-470 (30 mg/kg, s.c.) days 4, 6 and 8 and minocycline (10 mg/kg, i.p.) daily days 4–8 after tumor cell implantation (●)

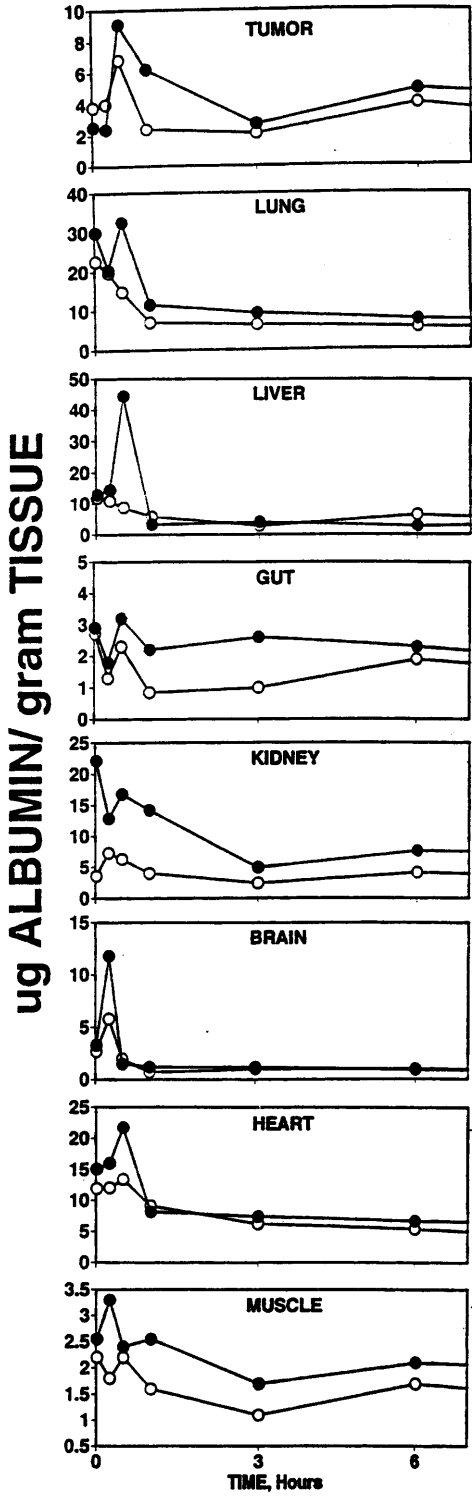

Fig. 3 Tissue levels of ^{14}C from [^{14}C]albumin in C57BL mice bearing Lewis lung tumors subcutaneously in the hind leg over time after intravenous injection of 10 mg/kg of the protein alone on day 8 (○) or after administration of the protein to animals treated with TNP-470 (30 mg/kg, s.c.) days 4, 6 and 8 and minocycline (10 mg/kg, i.p.) daily days 4–8 after tumor cell implantation (●)

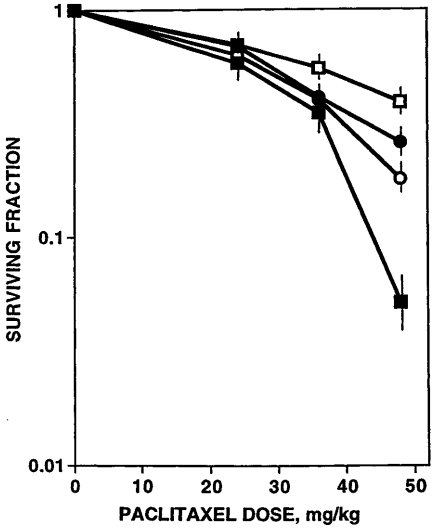

Fig. 4 Survival of EMT-6 tumor cells (●, ■) and bone marrow CFU-GM (○, □) from animals treated in vivo with single intravenous doses of paclitaxel alone on day 8 (●, ○) or after pretreatment with TNP-470 (30 mg/kg) and minocycline (10 mg/kg) on days 4 through 8 (■, □). Points are the means of three independent determinations; bars are the SEM

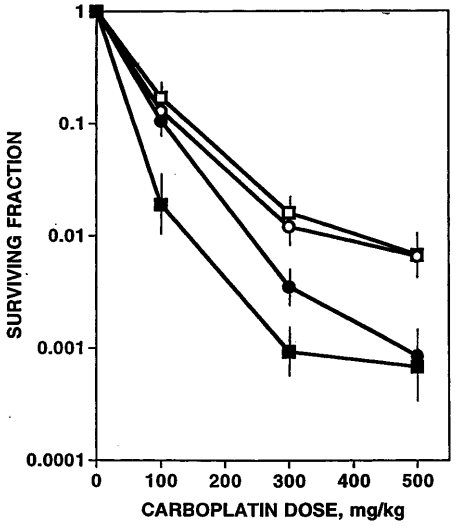

Fig. 5 Survival of EMT-6 tumor cells (●, ■) and bone marrow CFU-GM (○, □) from animals treated in vivo with single intraperitoneal doses of carboplatin alone on day 8 (●, ○) or after pretreatment with TNP-470 (30 mg/kg) and minocycline (10 mg/kg) on days 4 through 8 (■, □). Points are the means of three independent determinations; bars are the SEM

Administration of TNP-470/minocycline on days 4 through 8 along with single doses of carboplatin on day 8 resulted in increased EMT-6 tumor cell killing in animals receiving the lower two doses of carboplatin but not the highest dose of the drug (Fig. 5). There was 5.5-fold and 3.8-fold greater killing of EMT-6 tumor cells in animals pretreated with TNP-470/minocycline along with carboplatin at doses of 100 mg/kg and 300 mg/kg compared with animals receiving carboplatin alone, respectively. Pretreatment with TNP-470/minocycline had no effect on the toxicity of carboplatin toward the bone marrow CFU-GM.

Discussion

Despite numerous efforts to produce efficacious treatments, the outcome for patients with advanced non-small-cell lung cancer remains poor. Based on positive phase II clinical data the combination of paclitaxel and carboplatin has quickly become one of the most commonly used chemotherapeutic regimens for treatment of this disease. With this new therapy, clinical outcomes have clearly improved, but median survival in metastatic disease is still only approximately 12 months; without question a paradigm shift is needed.

Tumors are dynamic, complex, living tissues undergoing the varied processes of tissue growth under the guidance of aberrant malignant cells. Cytotoxic anticancer therapies have focused solely on the eradication of the malignant cell which is an absolute necessity in cancer therapy. The growth processes of tumors are normal processes; the invasion processes of tumors are normal processes; it is the inappropriate activation of these processes that comprises the morbidity of malignant disease. These normal processes involving normal cells are valid therapeutic targets. A systems approach to cancer therapy involves choosing multiple targets in malignant and normal cells for therapeutic attack to develop more effective therapeutic regimens [24].

The combination of TNP-470 and minocycline along with cytotoxic anticancer therapies forms a particularly effective treatment for the Lewis lung carcinoma [29]. Minocycline is a tetracycline and a matrix metalloproteinase inhibitor, specifically a collagenase inhibitor, which has demonstrated antiangiogenic activity [1, 23, 28–31, 33]. The characteristics of minocycline as a modulator of cytotoxic therapies in Lewis lung carcinoma have been described [1, 28]. Although exposure to TNP-470/minocycline along with cytotoxic anticancer agents in cell culture does not markedly increase cell killing by the cytotoxic agents [31], treatment of tumor-bearing animals with the same combination regimens markedly increases tumor growth delay and when Lewis lung carcinoma-bearing mice are treated with TNP-470/minocycline and cyclophosphamide, 40% to 50% of the animals are cured [29]. In vivo increased levels of drugs, cyclophosphamide and cisplatin are detected in the tumors of animals treated with TNP-470/minocycline [31].

The successful treatment of cancer requires the eradication of all malignant cells and, hence, treatment with cytotoxic therapies. The compatibility of antiangiogenic therapy with cytotoxic therapeutic treatments is not obvious. If the angiogenic and invasive characteristics of a tumor can be identified, that is expression of angiogenic factors and extracellular proteases, and the molecular targets of antiangiogenic agents elucidated then tumor-specific antiangiogenic therapeutic regimens

could be developed. Northern blot analysis has shown that Lewis lung carcinoma expresses high levels of basic fibroblast growth factor and low levels of vascular endothelial cell growth factor (bFGF:VEGF 10:1) in the absence of therapy [12]. After treatment with cytotoxic therapy, production of VEGF and transforming growth factor β (TGFβ) is markedly induced. Although the mechanism(s) of action of the antiangiogenic therapy, TNP-470/minocycline, have not been fully elucidated, it is most probable that each of these molecules has a different biologic target. As has been shown previously [30, 31] for [^{14}C]cyclophosphamide, cisplatin, Hoechst 33342 and molecular oxygen, administration of TNP-470/minocycline for several days prior to paclitaxel, carboplatin or labeled albumin results in higher concentrations of these molecules in the tumor. Unlike humans, rodents grow throughout their life and the 8- to 10-week-old animals used in these studies were rapidly growing. It is likely that because of this higher concentrations of each of the three molecules tested were found in other tissues in these animals.

The cytotoxic therapies, paclitaxel and carboplatin, were active antitumor agents against the Lewis lung carcinoma but were not highly effective. It is interesting that although there was some variation in the distribution of carboplatin into the various tissues examined, there was marked variation in the distribution of paclitaxel into the various tissues with the highest levels of that drug being found in lung followed by the gut and the heart. This tissue preference of the paclitaxel molecule may, in part, reflect its efficacy as an antitumor agent. Addition of the antiangiogenic therapy to each of the cytotoxic drugs alone and in combination improved the activity of the regimen. Perhaps the important factor in translating these findings to clinical studies is that the addition of antiangiogenic agents to treatment is most likely to make very good therapeutic regimens better.

The focus of this study has been on the therapeutic application of antiangiogenic agents to the treatment of non-small-cell lung cancer. The results in this in vivo model of an established solid tumor, as well as results from other systems including EMT-6 mammary carcinoma [33], the 9L gliosarcoma [32] and the FSaII fibrosarcoma [29], have shown that TNP-470/minocycline can interact in a very positive way with a variety of cytotoxic anticancer therapies and provide direction for future clinical trials.

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