Cancer Chemother Pharmacol (2003) 51: 43–52
DOI 10.1007/s00280-002-0529-0

ORIGINAL ARTICLE

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Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA)

Received: 18 July 2002 / Accepted: 2 September 2002 / Published online: 12 November 2002

Abstract *Purpose*: To determine whether there is a therapeutic interaction between the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and nine chemotherapy drugs against an early-passage mouse mammary tumour (MDAH-MCa-4), and to investigate the mechanism of any such interaction. *Methods and results*: Female C3H/HeN mice bearing intramuscular MDAH-MCa-4 tumours were injected intraperitoneally with DMXAA (80 μmol/kg) or chemotherapy drug (at a range up to the maximum tolerated dose) alone, or coadministered. A small reduction in the dose of the chemotherapy drug was required in most cases, but the increase in antitumour effect was much greater than the increase in host toxicity (body weight loss). The therapeutic gain increased in the order 5-fluorouracil (no gain) < (etoposide, carboplatin, cyclophosphamide, doxorubicin, cisplatin) < (docetaxel, vincristine) < paclitaxel. The interaction with paclitaxel (31.6 μmol/kg) was striking, with coadministration of DMXAA extending the median tumour growth delay from 0.3 to 80 days with three of seven animals cured. The interaction showed a broad timing of the optimum with similar activity when paclitaxel was administered 4 h before to 1 h after DMXAA. No therapeutic synergy was obtained when paclitaxel was combined with the antivascular agent combretastatin A4 phosphate (227 μmol/kg), which induced only transient blood flow inhibition in this tumour, measured using the H33342 perfusion marker. Paclitaxel did not enhance the antivascular activity of DMXAA. Plasma and tumour concentrations of paclitaxel (and carboplatin), measured by LC-MS and ICP-MS respectively, were not elevated by combination with DMXAA. *Conclusions*: There was a dramatic therapeutic interaction between DMXAA and standard chemotherapy drugs, particularly paclitaxel, against the MDAH-MCa-4 tumour, which was not due to a pharmacokinetic interaction or potentiation of antivascular activity. It is suggested that the major mechanism of synergy is killing of cells by DMXAA in poorly perfused regions of tumours that are inaccessible to chemotherapy drugs.

Keywords DMXAA · Combretastatin · Antivascular agents · Paclitaxel · Pharmacokinetics

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Introduction

The vascular system in tumours is a promising target for cancer therapy. A large number of antiangiogenic agents that inhibit the formation of new blood vessels in tumours have been identified, with several of these currently in clinical trial [14]. A complementary approach is provided by antivascular agents that damage existing blood vessels in tumours, leading to loss of blood flow and death of tumour cells dependent on the affected vessels. Antivascular agents capable of causing irreversible damage to tumour blood vessels include tumour necrosis factor [13, 30], tubulin binders such as vinblastine [2, 11] and combretastatin A4 phosphate (CA-4-P) [5, 7], flavone-8-acetic acid [4, 40] and its analogue 5,6-dimethylxanthenone-4-acetic acid (DMXAA) [17, 41], and arsenic trioxide [18]. Phase I clinical trials of DMXAA and CA-4-P have recently been completed (Jameson et al., Rustin et al., submitted for publication), with dynamic MRI showing

that both agents induce a significant reduction in tumour blood flow at well-tolerated doses [8, 9]. DMXAA and CA-4-P are thus the first antivascular agents for which activity (irreversible inhibition of tumour blood flow) has been documented in human tumours.

Preclinical studies with DMXAA and CA-4-P, using murine tumours or human tumour xenografts, have shown that their antivascular activity produces prolonged inhibition of tumour blood flow leading to extensive regions of haemorrhagic necrosis [17, 41]. However, tumours rapidly regrow from surviving cells in the well-perfused periphery [6, 17]. The transient tumour growth inhibition seen in most preclinical models may be consistent with similar activity documented in initial phase I clinical trials.

Despite this limited single-agent activity, there are sound theoretical reasons to expect that the clinical application of antivascular agents such as DMXAA will be in combination with conventional cytotoxic drugs, and with other modalities such as radiotherapy or hyperthermia. Not only are the dose-limiting toxicities of DMXAA (predominantly transient and reversible neurological disturbances; Jameson et al., Rustin et al., submitted for publication) distinct from those of most other anticancer agents, but importantly there are a number of mechanisms through which DMXAA can be expected to enhance the antitumour activity of other agents.

The first potential mechanism is that falling tumour blood flow can trap a second agent within the tumour, resulting in an increased tumour cell exposure. Such entrapment has been shown to contribute to enhancement by DMXAA of the antitumour activity of melphalan in preclinical models [26]. DMXAA similarly enhances radioimmunotherapy [24] and antibody-directed enzyme prodrug therapy [25] by increasing retention of the antibody conjugates in tumour xenografts. This inhibition of vascular washout of a second agent is analogous to the enhancement of hyperthermia by DMXAA through improvement of local heating by inhibiting blood flow [21].

A second mechanism is that microenvironmental changes secondary to inhibition of tumour blood flow (hypoxia, acidosis) could increase sensitivity to a second agent. DMXAA induces hypoxia in experimental tumours [32], and therapeutic benefit has been demonstrated from combining DMXAA with hypoxia-activated bioreductive drugs [6, 17, 37]. Both acidosis and hypoxia appear to contribute to the enhancement of melphalan activity by DMXAA [26].

Thirdly, the ability of DMXAA to cause necrosis selectively in the central regions of tumours suggests that it is ideally suited to complement agents which are less effective against poorly perfused regions of tumours. This complementarity is thought to account for the therapeutic gain obtained by combining DMXAA and radiation, demonstrated against four different murine tumours [22, 38]. Similar complementation can be expected with many conventional chemotherapy drugs likely to be less active in poorly perfused regions of tumours because of drug delivery problems, and/or resistance arising from cell cycle arrest. A fourth potential mechanism of interaction is through induction of cytokines and other bioactive species by DMXAA [1], which has the potential to alter intrinsic sensitivity of tumour cells to other agents.

The aim of the present study was to determine which chemotherapeutic agents might be most promising for combination with DMXAA. We surveyed nine cytotoxic drugs, representing the major classes used in the treatment of solid tumours, by investigating whether coadministration of DMXAA provided a therapeutic gain against established (0.6 g) early-passage mouse mammary carcinomas (line MDAH-MCa-4). Of the agents investigated the greatest enhancement, and greatest overall activity, was observed on combining DMXAA with paclitaxel. For this combination, we investigated the timing of administration of the two agents, and whether the therapeutic enhancement stemmed from a pharmacokinetic interaction or from enhanced antivascular activity. Whether CA-4-P provided similar synergy with paclitaxel was also investigated.

Materials and methods

Compounds

A stock solution of DMXAA, synthesized in the Auckland Cancer Society Research Centre, was prepared in phosphate-buffered saline, protected from light [27], and stored frozen. Cisplatin (Sigma, Mo.), CA-4-P (Oxigene Europe, Lund, Sweden), and Hoechst 33342 (Sigma) were dissolved in 0.9% saline. Stock solutions of carboplatin, 5-fluorouracil (Bristol-Myers Squibb, Sermonita, Italy) and cyclophosphamide (Mead Johnson Oncology Products, N.J.) were diluted with sterile water. Docetaxel (Rhone-Poulenc Rorer, Coubevouie, France), doxorubicin (Farmitalia Carlo Erba, Clayton North, Australia), etoposide and vincristine (Bristol-Myers Squibb) were diluted using 0.9% saline. Paclitaxel (Phytogen Life Sciences, Delta, BC, Canada) was stored frozen in ethanol, and diluted sequentially with Cremophor EL (1 vol) and 0.9% saline (10 vol) immediately before use. All compounds were administered to mice by intraperitoneal (i.p.) injection at 0.01 ml/g body weight. Cephalomannine (LC-MS internal standard) was obtained from the National Cancer Institute (MD, USA) and dissolved in dimethyl sulfoxide to 10 m*M*.

Animals and tumours

This study was approved by the Animal Ethics Committee at the University of Auckland. Murine mammary carcinoma MDAH-MCa-4 tumours [33] were grown from stocks stored in liquid nitrogen at the sixth transplant generation. Tumours (eighth transplant generation when used) were grown from 20 µl cell suspension (7 mg packed cells), inoculated intramuscularly into the right gastrocnemius muscle of female C_3H/HeN mice (22–25 g at the time of treatment). Mice were randomized to treatment which commenced when the tumour plus leg diameter reached 10–11 mm (about 0.6 g tumour) approximately 18 days after inoculation.

Host toxicity and antitumour activity

Mice were treated with chemotherapeutic drugs at a range of doses, at 1.33-fold increments, up to the expected maximum tolerated dose (MTD) (as estimated in pilot experiments or from the

literature). Toxicity was assessed as lethality, and body weight loss measured 5 days after treatment. Any animals becoming moribund or showing protracted clinical signs of toxicity were killed and treated as drug-related deaths in the analysis. The diameter of the tumour-bearing leg was measured three times per week after treatment. Antitumour activity was assessed from the tumour growth delay, defined as the difference in time to reach the endpoint of 13 mm (1.5 g tumour) for treated and control groups. Responses were classed as cures if animals were free of evident tumour 120 days after treatment. The statistical significance of tumour growth inhibition was tested by ANOVA using SAS for Windows, with Dunnett's test to evaluate *P*-values for differences between treatment groups. In experiments with a substantial numbers of cures, statistical significance was tested by Kruskal-Wallis non-parametric analysis of variance using SAS for Windows, and the difference between treatment and control groups by Dunn's test using Sigmastat v2.0. The gradient and standard error of dose-response curves was determined by linear regression and the dose modifying factor (DMF) calculated as the gradient with DMXAA/gradient without DMXAA.

Tumour blood flow inhibition

Tumour blood flow was assessed using the fluorescent perfusion marker Hoechst 33342 (8 mg/ml in saline), which was administered intravenously at various times after drug treatment. Mice were killed 2 min later, and frozen sections (14 μm) prepared from the distal, central and proximal regions of each tumour. Sections were examined with a Nikon epifluorescence microscope at ×10 magnification using a UV-1A filter block (excitation 365 nm, barrier filter 400 nm, and dichroic mirror 400 nm). A grid with 81 squares (100×100 μm) was used for point scoring of staining. The whole area of each section was scored to avoid bias between peripheral and central regions (which were less-well perfused). Normal tissue was excluded but necrotic areas were included. Differences between groups were tested for significance using Student's *t*-test (Sigmastat v2.0).

Pharmacokinetics

Female C_3H/HeN mice bearing MDAH-MCa-4 tumours (about 0.6 g) were injected i.p. with carboplatin (316 μmol/kg) or paclitaxel (23.7 μmol/kg) alone, or simultaneously with DMXAA (80 μmol/kg). At various times blood was collected from the retro-orbital sinus of anaesthetized mice into heparinized tubes, and the plasma separated by centrifugation. Mice were killed while still under anaesthesia after each bleed. Tumours were rapidly dissected and frozen at –80°C. Plasma and tumour samples from mice that had received combination treatment were divided into two for separate analysis of DMXAA and carboplatin or paclitaxel. Groups of two to five mice were used for each time-point.

ICP-MS analysis of platinum

Concentrations of platinum in plasma and tumours were determined using a previously published ICP-MS method [28, 29]. Briefly, tumours were digested in 70% nitric acid (2 h, 90°C) and diluted in water, while plasma was deproteinized with an equal volume of methanol and diluted in 0.1% nitric acid. Samples were analysed with an Agilent 4500 ICP-MS (Agilent Technologies, Waldbronn, Germany) with a nickel sampling cone, Babington (v-groove) nebulizer and a Scott double-pass spray chamber maintained at 2°C. Platinum was read at 195 amu with a dwell time of 100 ms and a replicate time of 6000 ms. Other operating conditions and intraassay and interassay variability and recovery were as previously reported [28]. Calibration curves were linear ($r^2 > 0.98$) over a wide range (0.5 to 5000 ng/ml). The limits of quantitation were 20 ng platinum per ml plasma and 10 ng platinum per g tumour tissue.

LC-MS analysis of paclitaxel

Concentrations of paclitaxel in plasma and tumour were determined by HPLC with detection by positive mode electrospray mass spectrometry. Aliquots of plasma (50 μl) were treated with 1 ml ice-cold acetonitrile/methanol (3:1 v/v) to precipitate proteins. Following addition of 5 μl of an internal standard solution (10 m*M* cephalomannine), samples were centrifuged, and the supernatants evaporated using a Speed-Vac solvent concentrator (Savant Instruments, Holbrook, N.Y.). The residues were dissolved in 50 μl methanol plus an equal volume of mobile phase (48% acetonitrile in 1 m*M* sodium acetate buffer, pH 6.2), and 5–40 μl analysed by LC-MS. Frozen tumours were minced, then homogenized in 2 vol distilled water using a Polytron homogenizer. Ice-cold acetonitrile (1 ml), and 10 μl internal standard (10 m*M* cephalomannine) were added to 200 μl tumour homogenate. The samples were centrifuged and the supernatants dried as above. The residues were dissolved in 100 μl methanol plus 100 μl mobile phase, and 5 μl was analysed by LC-MS.

The LC-MS system was an Agilent 1100/MSD (Agilent Technologies, Waldbronn, Germany) with a 3.2×150 mm C_8 5-μm column (Alltima Associates, Deerfield, Ill.) and a flow rate of 0.7 ml/min. The mass detector used N_2 as the nebulizing and drying gas (flow rate 10 l/min, 350°C; nebulizer pressure 25 psi, capillary voltage 2500 V, fragmenter voltage 120 V). Paclitaxel (MW 854) and cephalomannine internal standard (MW 832) were detected, using single ion monitoring, as sodium adducts at m/z 876 and 854, respectively. Spiking of control material indicated assay linearity in the range 0.3–30 μ*M* for both plasma and tumour ($r^2 = 0.999$). The intraassay and interassay precision and accuracy gave coefficients of variation $\leq 5\%$, and recoveries in the range 92–102%. The lower limit of detection of paclitaxel in plasma and tumour (signal:noise ratio of 3) was 0.3 μ*M*.

HPLC analysis of DMXAA

Concentrations of DMXAA in plasma were determined using a modification of a published method [15]. Aliquots of plasma (50 μl) were treated with 1 ml ice-cold acetonitrile/methanol (3:1 v/v), centrifuged, and the resulting supernatants evaporated as above. The residues were dissolved in 200 μl 10 m*M* ammonium acetate buffer (pH 5) and 25 μl was analysed by HPLC using a HP1100 system with diode array detector (278 nm) and fluorescence detector (excitation 242 nm, emission 396 nm). The column and flow rate were as for paclitaxel above, with a mobile phase of 16% acetonitrile (v/v) in 10 m*M* ammonium acetate buffer (pH 5). Spiking of control plasma showed assay linearity in the range 0.1–100 μ*M* ($r^2 = 0.999$). The intraassay and interassay precision and accuracy gave coefficients of variation $< 7\%$, and an average recovery of 70%. The lower sensitivity limit of detection by fluorescence (signal:noise ratio of 3) was 0.1 μ*M*.

Pharmacokinetic modelling

Pharmacokinetic data were modelled using ModelMaker version 4.0 (Cherwell Scientific, Oxford, UK). For each compound it was assumed that all of the administered dose reached the central compartment (i.e. 100% bioavailability). Differences between treatment groups were tested using an *F*-test comparing the entire curves, and if this difference was significant ($P \leq 0.05$) the estimates of each individual model parameter for both groups were tested using a two-tailed *t*-test. The concentrations of free platinum and total platinum in plasma and tumour, respectively, of mice treated with carboplatin were fitted using a two-compartment open model assuming linear pharmacokinetics. The concentrations of paclitaxel in plasma and tumour were fitted using a one-compartment open model assuming linear pharmacokinetics (a two-compartment model for the tumour data was rejected based on an *F*-test). Plasma concentrations of DMXAA were fitted using a one-compartment open model with saturable (Michaelis-Menten) elimination

kinetics. Noncompartmental analysis was performed with WinNonlin version 3.1 (Pharsight Corporation, Mountain View, Calif.).

Results

Activity of DMXAA plus chemotherapy drugs against the MDAH-MCa-4 tumour

The antitumour activities and host toxicity of DMXAA/cytotoxic drug combinations were assessed by varying the dose of chemotherapeutic drug up to the toxicity limit, with coadministration of a fixed DMXAA dose (80 μmol/kg or 24 mg/kg, about 80% of the MTD), and evaluating subsequent tumour growth delay, as illustrated in Fig. 1. Of the nine drugs investigated, four (5-fluorouracil, cisplatin, doxorubicin and cyclophosphamide) showed appreciable single-agent activity against this tumour as indicated by dose-response relationships with significant positive gradients by linear regression, and highly significant growth delays of about 10 days at their MTDs (which are recorded in Table 1). The other five compounds (carboplatin, vincristine, etoposide, docetaxel and paclitaxel) were essentially inactive, with no individual treatment groups showing significant activity (although carboplatin and paclitaxel gave weakly positive dose responses by linear regression).

DMXAA alone showed activity as a single agent at 80 μmol/kg, providing transient regressions and mean tumour growth delays in the range 3.5–13.1 days over a series of experiments (overall mean 7.9 ± 0.8 days). This activity was statistically significant ($P < 0.05$) in eight of the nine experiments. Coadministration of DMXAA at this dose increased the host toxicity of docetaxel,

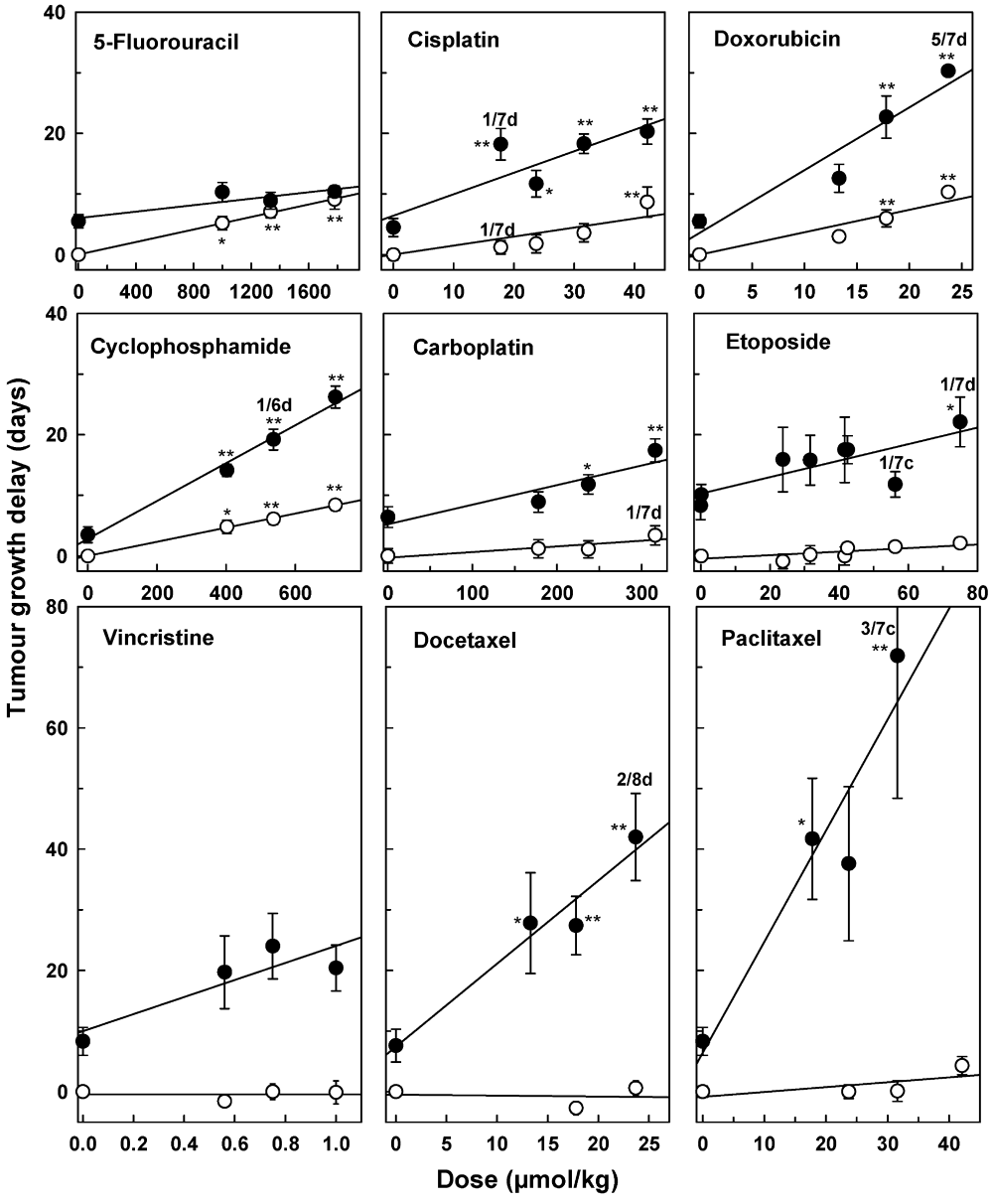

Fig. 1 Growth delay of intramuscular MDAH-MCa-4 tumours after treatment of mice with chemotherapeutic drug i.p. alone (*open symbols*) or coadministration of drug with i.p. DMXAA at 80 μmol/kg (*filled symbols*). Values are means ± SEM for groups of six to eight mice, ignoring deaths (*d*) or cures (*c*), the numbers of which are shown. $*P < 0.05$, $**P < 0.01$, significance of growth delay relative to corresponding control (no chemotherapy drug)

Table 1 Effect of DMXAA on host toxicity and antitumour activity of chemotherapeutic drugs against MDAH-MCa-4 tumours. Drugs were coadministered with DMXAA by i.p. injection (all errors are SEM)

Chemotherapy drug	DMXAA (μmol/kg)	MTD (μmol/kg)	Body weight change at 5 days (%)	Slope of dose/response (days/μmol/kg)	DMF	Median growth delay at MTD (days)
DMXAA	80	100	2.4 ± 0.7			6.7[a]
5-Fluorouracil	–	1780	–8.5 ± 0.8	0.0051 ± 0.0001		10.9
	80	1780	–14.1 ± 1.5	0.0027 ± 0.001	0.5 ± 0.2	10.7
Cisplatin	–	42.1	–9.5 ± 1.4	0.19 ± 0.06		7.1
	80	42.1	–14.4 ± 2.3	0.35 ± 0.12	1.8 ± 1.2	20
Doxorubicin	–	23.7	–3.9 ± 0.6	0.42 ± 0.10		9.3
	80	17.8	–5.5 ± 1.2	1.04 ± 0.23	2.5 ± 1.1	22.2
Cyclophosphamide	–	≥716	–0.8 ± 1.3	0.0062 ± 0.0001		7.7
	80	≥716	–9.2 ± 1.4	0.0167 ± 0.0013	2.7 ± 0.3	28
Carboplatin[b]	–	316	–5.6 ± 1.5	0.0094 ± 0.0035		1.0
	80	316	–8.5 ± 1.4	0.032 ± 0.010	3.4 ± 2.3	14.9
Etoposide	–	≥75	–2.0 ± 1.7	0.030 ± 0.010		1.5
	80	75	–6.5 ± 3.3	0.14 ± 0.04	4.7 ± 2.9	20.5
Vincristine	–	1.0	–7.0 ± 1.2	–0.0 ± 1.3		1.0
	80	1.0	–10.0 ± 1.4	14.1 ± 5.4	> 7[c]	17.3
Docetaxel	–	23.7	–4.1 ± 1.3	–0.02 ± 0.14		–0.2
	80	17.8	–7.2 ± 1.2	1.37 ± 0.22	> 9[c]	42.1
Paclitaxel	–	42.1	0.3 ± 1.1	0.08 ± 0.06		4.4
	80	31.6	–3.9 ± 1.2	1.83 ± 0.4	> 13[c]	80

[a]For DMXAA dose 80 μmol/kg, data from nine experiments
[b]In a limited repeat experiment, significant toxicity was observed at 316 μmol/kg (three deaths among seven animals without DMXAA and one death among seven animals with DMXAA), and the combination with DMXAA was only additive (DMF 1.0)
[c]Estimated using upper error estimate of the slope for the chemotherapy drug only

doxorubicin and paclitaxel; in each case the MTD for the chemotherapy drug was lowered by one dose level (1.33-fold) in the combination (Table 1). For the other compounds, coadministration of DMXAA did not require dose reduction although some additional toxicity was evident as indicated by the greater body weight loss with the combination (Table 1).

In contrast to this small effect on host toxicity, coadministration of DMXAA produced a large enhancement of tumour growth delay with most of the cytotoxic drugs (Fig. 1, Table 1). The contribution of DMXAA to the tumour response was assessed by determining the slope of each dose-response curve by linear regression, with the DMF for DMXAA calculated as the ratio of the slopes with and without DMXAA. By this criterion, the magnitude of the synergy increased in the order 5-fluorouracil (therapeutic antagonism) < (cisplatin, doxorubicin, cyclophosphamide, carboplatin, etoposide) < (vincristine, docetaxel) < paclitaxel. As an alternative criterion, the maximum tumour growth delay achievable at the MTD of the combination indicated the taxanes to be the most active compounds in combination with DMXAA. All the other compounds (except 5-fluorouracil) also provided strong activity in the combination, with growth delays in the range 15–30 days. Individual tumour growth curves are illustrated for groups from the experiment in which paclitaxel was combined with DMXAA (Fig. 2), demonstrating that the antivascular agent alone caused transient regressions while the combination caused variable but marked responses (including three of seven mice cured).

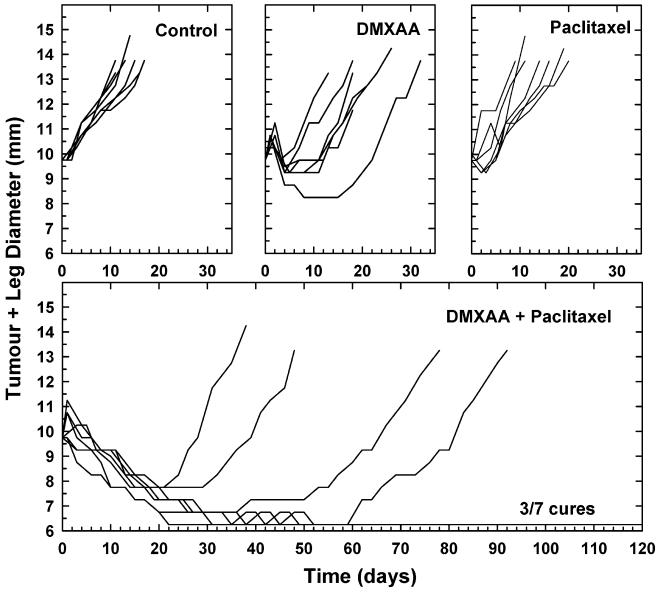

Fig. 2 Individual growth curves for intramuscular MDAH-MCa-4 tumours following treatment with DMXAA (80 μmol/kg), paclitaxel (31.6 μmol/kg), or coadministered DMXAA plus paclitaxel

DMXAA plus paclitaxel: timing and dose-response relationship

The interaction between DMXAA and paclitaxel was examined in further detail. Repetition of the experiment illustrated in Fig. 1 confirmed the dramatic activity of this combination, in this case leading to three of seven

mice cured at a paclitaxel dose of 23.7 μmol/kg and one of seven mice cured at 31.6 μmol/kg. One drug-related death was observed in each of these groups, but body weight loss was greater at the higher dose (4.3 ± 1.8% versus 7.4 ± 1.8% respectively). The lower paclitaxel dose (23.7 μmol/kg) was used in all further studies.

The time of administration of the two agents was varied, with paclitaxel (23.7 μmol/kg) administered up to 4 h before or after DMXAA (Fig. 3). Substantial activity was seen in most groups, with two or three cures per group of 12 mice and large tumour growth delays (median about 80 days) for the recurring tumours. The exception was DMXAA 4 h before paclitaxel which resulted in no cures and a smaller growth delay which, because of the large variability, was below the level of statistical significance. In each group (including coadministration) the toxicity of the combination was higher than in the previous coadministration experiments, with two or three deaths per 12 mice and mean body weight loss in the range 6–10%.

Given the appreciable toxicity of the DMXAA/paclitaxel combinations, the DMXAA dose-response relationship was also examined (Fig. 4). As in the other experiments, paclitaxel alone at 23.7 μmol/kg had no significant activity, but was significantly enhanced by DMXAA at all three dose levels tested. DMXAA showed a steep dose-response relationship in the combination, with a threshold at a DMXAA dose of approximately 50 μmol/kg. In this experiment the combination of paclitaxel (23.7 μmol/kg) with DMXAA at 80 μmol/kg was highly toxic (five deaths among 12 mice) with one cure among the seven remaining animals, although body weight loss (mean 7.6 ± 2.9%) was in the usual range for this dose level and was no higher than for DMXAA alone (7.6 ± 1.6%).

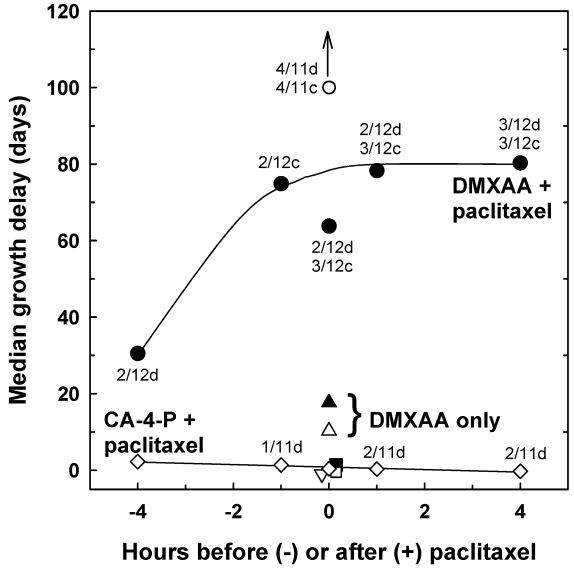

Fig. 3 Effect of varying the timing of DMXAA (80 μmol/kg) or CA-4-P (227 μmol/kg) relative to paclitaxel (23.7 μmol/kg) on activity against MDAH-MCa-4 tumours. Data are pooled from two experiments: *filled symbols* experiment 1, *open symbols* experiment 2; *up triangles* DMXAA alone, *squares* paclitaxel alone, *down triangle* CA-4-P alone, *circles* DMXAA plus paclitaxel, *diamonds* CA-4-P plus paclitaxel. The growth delays are shown as the medians because of the high proportions of cures (*c*) and treatment-related deaths (*d*), the numbers of which are shown

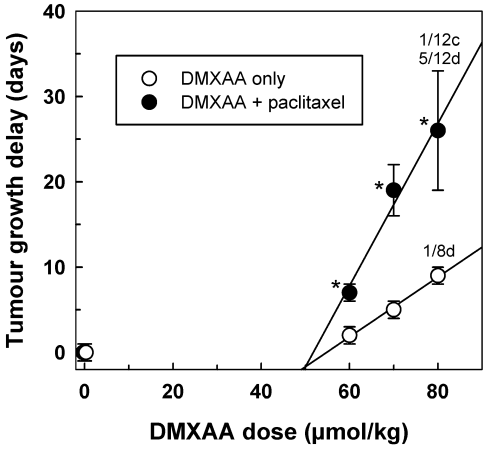

Fig. 4 DMXAA dose dependence of growth inhibition of MDAH-MCa-4 tumours in mice treated with DMXAA alone or in combination with paclitaxel (23.7 μmol/kg). Values are means ± SEM for 8 (DMXAA only) or 12 (DMXAA plus paclitaxel) animals. $*P < 0.05$, activity of the combination significantly greater than DMXAA alone

Activity of CA-4-P plus paclitaxel against the MDAH-MCa-4 tumour

Given the striking interaction between DMXAA and paclitaxel, we investigated whether similar therapeutic synergy occurred with CA-4-P, an antivascular agent with a different mechanism of action which has also recently completed phase I clinical trials [5, 7]. As shown in Fig. 3, CA-4-P at 227 μmol/kg lacked activity against the MDAH-MCa-4 tumour. Administration of CA-4-P before, with, or after paclitaxel (23.7 μmol/kg) failed to elicit significant antitumour activity. In the same experiment, coadministration of DMXAA (80 μmol/kg) with paclitaxel led to four cures among 11 animals with four deaths due to toxicity.

Antivascular activity of CA-4-P and DMXAA, and effect of paclitaxel coadministration

The abilities of DMXAA and CA-4-P to inhibit blood flow in the MDAH-MCa-4 tumour were compared using H33342 as a fluorescent perfusion marker (Fig. 5A). DMXAA at 80 μmol/kg caused prompt and irreversible blood flow shutdown, with no recovery by 24 h. CA-4-P also inhibited tumour blood flow at 4 h, but this had reversed by 24 h. Whether paclitaxel enhanced the vascular shutdown by DMXAA at 24 h was assessed in a separate experiment, using a lower DMXAA dose (70 μmol/kg) so that any increased activity could be

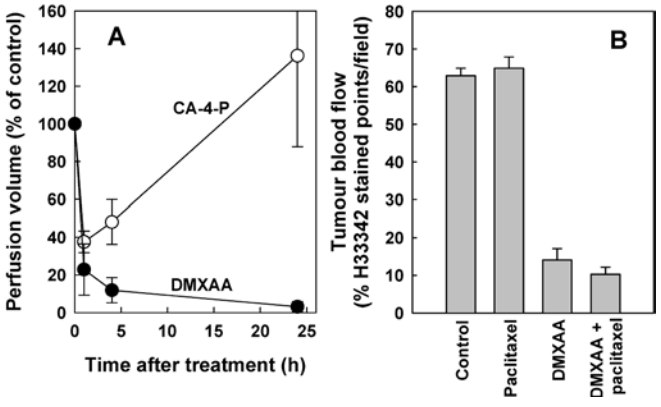

Fig. 5A, B Antivascular activity of DMXAA and CA-4-P against the MDAH-MCa-4 tumour determined by scoring H33342 perfusion volume by point counting. **A** Time-course of inhibition of perfusion following treatment with DMXAA (80 μmol/kg) (*closed circles*) or CA-4-P (227 μmol/kg) (*open circles*). Points are means ± SEM for three to five tumours. **B** Tumour perfusion 4 h after treatment with DMXAA (70 μmol/kg), paclitaxel (23.7 μmol/kg) or coadministration of the two drugs. Points are means ± SEM for five to seven tumours

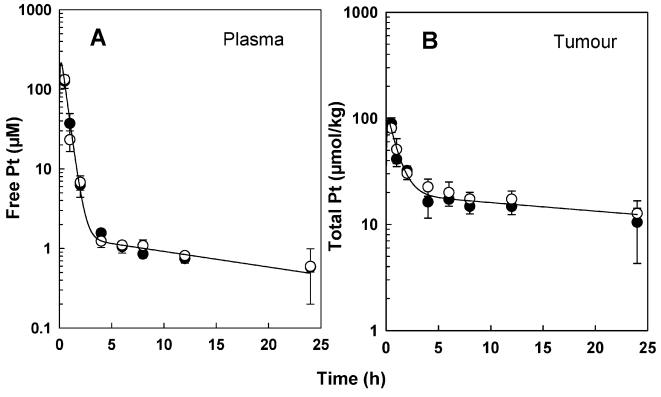

Fig. 6 **A** Plasma concentrations of platinum, after deproteinization, following administration of carboplatin alone (316 μmol/kg) (*closed circles*) or with DMXAA (80 μmol/kg) (*open circles*). **B** Tumour concentrations of total platinum following administration of carboplatin alone (*closed circles*) or with DMXAA (*open circles*). Lines are fits to a two-compartment open model assuming linear pharmacokinetics

more readily detected (Fig. 5B). This showed no antivascular activity of paclitaxel alone, and no significant increase in activity of the combination relative to DMXAA alone.

Pharmacokinetics of DMXAA plus carboplatin, and DMXAA plus paclitaxel

We determined whether a pharmacokinetic interaction underlies the beneficial therapeutic interaction between DMXAA and paclitaxel, and also whether such interactions contribute to the synergy between DMXAA and carboplatin. These studies were conducted in mice bearing MDAH-MCa-4 tumours of the same size as in the therapeutic studies. Following administration of carboplatin (316 μmol/kg, i.p.), clearance of platinum from plasma (measured, after deproteinization, by ICP-MS) was biphasic, and was unaffected by coadministration of DMXAA (Fig. 6A, Table 2). Total platinum in the tumour also showed biphasic kinetics, and again was unaffected by coadministration of DMXAA (Fig. 6B, Table 2).

A similar study was undertaken with the paclitaxel/DMXAA combination by comparing the pharmacokinetics of both agents alone and after coadministration (Fig. 7, Table 2). DMXAA was measured by HPLC using fluorescence detection (Fig. 7A), and paclitaxel by LC-MS (Fig. 7B, C). The latter employed electrospray mass spectrometry detection with a high sodium ion concentration in the mobile phase to suppress fragmentation, providing the $[M + Na]^+$ molecular ion (m/z 876), with cephalomannine (m/z 854) as internal standard. DMXAA clearance from plasma was unaffected by coadministration of paclitaxel at 23.7 μmol/kg (Fig. 7A). Similarly, DMXAA coadministration had no effect on plasma concentrations of paclitaxel (Fig. 7B). Paclitaxel concentrations in tumours at early times appeared to be slightly lowered by DMXAA coadministration and the apparent volume of distribution of paclitaxel in the tumour was increased (Fig. 7C,

Table 2 Non-compartmental pharmacokinetic parameters for carboplatin (316 μmol/kg) and paclitaxel (23.7 μmol/kg), alone and in combination with coadministered DMXAA (80 μmol/kg) in plasma and tumours of female C_3H/HeN mice bearing MDAH-MCa-4 tumours (T_{max} time of maximum observed concentration, C_{max} concentration corresponding to T_{max}, $(t_{1/2})_{\lambda z}$ terminal half-life, *AUC* area under the curve calculated using the linear/log trapezoidal rule and extrapolation to infinity, V_z volume of distribution based on the terminal phase, *Cl* total body or tumour clearance)

Parameter	Plasma						Tumour			
	Carboplatin		Paclitaxel		DMXAA		Carboplatin		Paclitaxel	
	Without DMXAA	With DMXAA	Without DMXAA	With DMXAA	Without paclitaxel	With paclitaxel	Without DMXAA	With DMXAA	Without DMXAA	With DMXAA
T_{max} (h)	0.5	0.5	2	4	0.5	0.5	0.5	0.5	6	6
C_{max} (μ*M* or μmol/kg)	127	132	4.3	5.4	459	499	90	81	2.2	1.3
$(t_{1/2})_{\lambda z}$ (h)	17	19	2.2	1.5	2.1	2.6	27	30	17	21
AUC (μ*M*·h)	122	117	21.0	24.3	3247	2830	822	1022	51.3	37.0
V_z (l/kg)	63	74	3.6	2.1	0.075	0.10	15.1	13.4	11.2	19.0
Cl (l/h/kg)	2.6	2.7	1.1	0.98	0.025	0.028	0.38	0.31	0.46	0.64

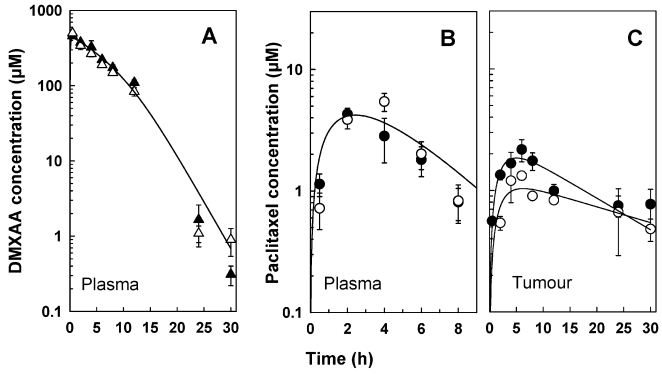

Fig. 7 **A** Plasma concentrations of DMXAA following administration of DMXAA (80 μmol/kg) alone (*open triangles*) or with paclitaxel (23.7 μmol/kg) (*open triangles*). **B** Plasma concentrations of paclitaxel following administration of paclitaxel alone (*closed circles*) or with DMXAA (*open circles*). **C** Tumour concentrations of paclitaxel following administration of paclitaxel alone (*closed circles*) or with DMXAA (*open circles*). Lines are fits to a one-compartment open model assuming linear pharmacokinetics (paclitaxel) or to a one-compartment open model with saturable (Michaelis-Menten) elimination kinetics (DMXAA)

Table 2). This change in paclitaxel concentrations in tumours is in the wrong direction to account for the enhanced antitumour activity of the combination.

Discussion

The hypothesis that antivascular agents such as DMXAA have the potential to combine synergistically with conventional cytotoxic agents in the treatment of solid tumours was tested in this study. The early-passage mammary tumour MDAH-MCa-4 used for this comparative study was moderately sensitive to most of the cytotoxic drugs tested (using single drug doses) showing significant responses to doxorubicin, 5-fluorouracil, cyclophosphamide and cisplatin and slight sensitivity to paclitaxel at high doses. DMXAA alone showed consistent activity as a single agent, of similar magnitude to the most active of the chemotherapy drugs, but none of the single agents provided prolonged tumour regressions or cures.

However, coadministration of DMXAA with the cytotoxic drugs caused a marked increase in response (Fig. 1). This interaction can be classified as synergistic on the basis of the increased slope of the cytotoxic drug dose response curve on addition of DMXAA. The interaction, quantified as the DMF (ratio of the linear regression slopes with and without DMXAA), was significantly greater than unity for all drugs except 5-fluorouracil. The therapeutic activity of DMXAA with cyclophosphamide or cisplatin in this study is consistent with a recent report of additive or supraadditive activity of DMXAA or CA-4-P with these drugs in three tumour models [31]. Of the nine drugs evaluated in the present study, the greatest potentiation of antitumour activity was observed for the taxanes docetaxel and paclitaxel. MDAH-MCa-4 tumours have been reported to be moderately sensitive to paclitaxel as a single agent with a single dose of 40 mg/kg (47 μmol/kg) producing a growth delay of 4.0 days ($P < 0.01$) [12], which is not inconsistent with our data (Fig. 1).

Combining DMXAA and paclitaxel produced significant antitumour activity in each of the experiments in the present study, although the magnitude of the activity (and toxicity) varied for reasons that are not known. Of 50 MDAH-MCa-4 tumour-bearing mice receiving coadministered DMXAA (80 μmol/kg) and paclitaxel (23.7 μmol/kg), 13 died from drug toxicity, while tumours were cured in 12, with an overall median growth delay of 44 days. The combination treatment was well tolerated at a lower DMXAA dose of 70 μmol/kg (no deaths among 12 animals) with significant antitumour activity (median growth delay 17.7 days) still obtained. In clinical evaluation it would be appropriate to escalate doses of DMXAA and paclitaxel with care because of the possibility of toxicity as well as therapeutic interactions.

Investigation of the timing of administration of DMXAA relative to paclitaxel indicated a broad optimum during which antitumour activity was maintained when paclitaxel was administered up to 4 h before or 1 h after DMXAA (Fig. 3). Administration of paclitaxel 4 h after DMXAA resulted in a substantial loss of antitumor activity, presumably as a result of decreased tumour blood flow inhibiting paclitaxel delivery. This is consistent with the data shown in Fig. 5A which shows that DMXAA treatment produced a rapid decrease in blood flow in MDAH-MCa-4 tumours, to levels $12 \pm 6\%$ of controls 4 h after administration. These results suggest that timing will be a critical consideration in designing schedules of DMXAA/chemotherapy combination treatments for clinical studies. The chemotherapy drug should be administered before, or shortly after the antivascular agent DMXAA in order to avoid compromised delivery.

CA-4-P produced a rapid but transient inhibition of blood flow in MDAH-MCa-4 tumours, with recovery to control levels by 24 h. The antivascular activity of CA-4-P appears to vary considerably between different tumours. The present results are similar to those of other studies in which a C_3H mammary carcinoma was used where CA-4-P produced a 65% inhibition of tumour blood perfusion 1 h after administration which had returned to control levels by 24 h, while DMXAA gave prolonged blood flow inhibition in the same model [20]. Similarly, blood flow in the human colon adenocarcinoma HT29 is sensitive to DMXAA but not CA-4-P, while both agents inhibit blood flow in the LS174T adenocarcinoma [3]. Although not equally active against all tumours, an advantage with CA-4-P is its broad dose response for antivascular activity in contrast to the steep DMXAA dose-response [7, 31, 32]. The lack of antitumour activity obtained from combining CA-4-P and paclitaxel in the present study suggests that irreversible vascular damage, as observed with DMXAA (Fig. 5A),

is required for potentiation of paclitaxel activity, although we cannot exclude the possibility of pharmacological antagonism between microtubule stabilization (paclitaxel) and destabilization (CA-4-P). It is also possible that there are interactions between DMXAA (TNF inducer) and paclitaxel (TNF agonist) at the TNF receptor level, although this is not supported by the similar synergistic antitumour activity with docetaxel (which lacks TNF agonist activity [19]).

The present study showed that the mechanism of antitumour synergy between DMXAA and paclitaxel does not involve potentiation of the antivascular activity of DMXAA or a pharmacokinetic interaction between the two drugs; the latter was also excluded as the basis for the interaction with carboplatin. This is in contrast to studies combining DMXAA and thalidomide in which coadministration resulted in a significant increase in the area under the concentration-time curve (AUC) of DMXAA in plasma and tumour [15]. Combining DMXAA and cyproheptadine also resulted in increased DMXAA half-lives in plasma and tumour tissue [39]. The present study also contrasts with the interaction between DMXAA and melphalan: although there was no change in systemic pharmacokinetics, tumour concentrations of melphalan were increased by pretreatment with DMXAA (interpreted as entrapment resulting from falling blood flow) [26]. The converse was observed with paclitaxel, with tumour levels lowered when DMXAA was coadministered resulting in a reduction in tumour AUC of about one-third. The latter effect could be modelled as an increase in the apparent volume of distribution. This might reflect an averaged tumour concentration that has been lowered by closure of some vessels by DMXAA, or lowering of paclitaxel concentrations at early times because of slowed uptake. In any event, the effect is in the wrong direction to account for the observed therapeutic synergy. Similar results have been reported for the combination of CA-4-P and 5-fluorouracil which did not change plasma pharmacokinetics but tumour AUC for 5-fluorouracil was significantly lowered by the antivascular agent [10].

A possible mechanism that could account for the increased antitumour activity is that DMXAA and paclitaxel have complementary activity against different subregions of the tumour, with DMXAA being more active in the poorly vascularized regions and paclitaxel more active in the well vascularized regions. This mechanism is thought to be responsible for the synergy obtained by combining DMXAA and radiation against the RIF-1 and MDAH-MCa-4 tumours [38] and for the interaction of DMXAA or CA-4-P with cisplatin and cyclophosphamide against murine tumours and human tumour xenografts [31]. Evidence to support this interpretation comes from histological examination of DMXAA-treated tumours which shows extensive haemorrhagic necrosis through the central region of the tumour, surrounded by a rim of viable tissue [17, 42]. Extravascular transport of paclitaxel is reported to be inefficient because of a binding site barrier [16, 23, 35] which is likely to lead to sparing of cells in poorly perfused regions. In addition, as a cycle-selective cytotoxin, paclitaxel would be expected to be less active against slowly cycling or noncycling cells in poorly perfused regions.

Complementary killing of different regions of tumours is potentially a very general mechanism of interaction with antivascular drugs, given that many conventional cytotoxins have limited activity against poorly perfused regions in tumours [34, 36]. As the taxanes have an important role in chemotherapy of breast, lung, head and neck, and ovarian cancers, and DMXAA has demonstrated good tolerability in recent phase I clinical trials, the present study suggests that clinical evaluation of their combination is warranted. We are currently investigating whether the dramatic synergy between DMXAA and standard chemotherapy drugs, including the taxanes, observed in the MDAH-MCa-4 mammary tumour carries over to other tumour models. Recent results have also demonstrated a synergistic interaction between DMXAA and paclitaxel in OCa-1 murine ovarian carcinomas and WiDr human colon carcinoma xenografts.

Acknowledgements This study was funded by Grant 81386 from the Auckland Medical Research Foundation, and a fellowship from the Health Research Council of New Zealand (W.R. Wilson).

References

1. Baguley BC, Ching LM (1997) Immunomodulatory actions of xanthenone anticancer drugs. Biodrugs 8:119
2. Baguley BC, Holdaway KM, Thomsen LL, Zhuang L, Zwi LJ (1991) Inhibition of growth of colon 38 adenocarcinoma by vinblastine and colchicine: evidence for a vascular mechanism. Eur J Cancer 27:482
3. Beauregard DA, Pedley RB, Hill SA, Brindle KM (2002) Differential sensitivity of two adenocarcinoma xenografts to the anti-vascular drugs combretastatin A4 phosphate and 5,6-dimethylxanthenone-4-acetic acid, assessed using MRI and MRS. NMR Biomed 15:99
4. Bibby MC, Double JA, Loadman PM, Duke CV (1989) Reduction of tumor blood flow by flavone acetic acid: a possible component of therapy. J Natl Cancer Inst 81:216
5. Chaplin DJ, Pettit GR, Hill SA (1999) Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. Anticancer Res 19:189
6. Cliffe S, Taylor ML, Rutland M, Baguley BC, Hill RP, Wilson WR (1994) Combining bioreductive drugs (SR 4233 or SN 23862) with the vasoactive agents flavone acetic acid or 5,6-dimethylxanthenone acetic acid. Int J Radiat Oncol Biol Phys 29:373
7. Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ (1997) Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. Cancer Res 57:1829
8. Galbraith SM, Lodge MA, Taylor NJ, Maxwell RJ, Tozer GM, Prise VE, Wilson I, Sena A, Robbins A, Padhani A, Rustin G (2001) Combretastatin A4 phosphate (CA4P) reduces tumor blood flow in animals and man, demonstrated by MRI. Proc Am Soc Clin Oncol 20:278a
9. Galbraith SM, Rustin GJ, Lodge MA, Taylor NJ, Stirling JJ, Jameson M, Thompson P, Hough D, Gumbrell L, Padhani AR (2002) Effects of 5,6-dimethylxanthenone-4-acetic acid on human tumor microcirculation assessed by dynamic contrast-enhanced magnetic resonance imaging. J Clin Oncol 20: 3826

10. Grosios K, Loadman PM, Swaine DJ, Pettit GR, Bibby MC (2000) Combination chemotherapy with combretastatin A-4 phosphate and 5-fluorouracil in an experimental murine colon adenocarcinoma. Anticancer Res 20:229
11. Hill SA, Lonergan SJ, Denekamp J, Chaplin DJ (1993) Vinca alkaloids: anti-vascular effects in a murine tumour. Eur J Cancer 29A:1320
12. Inoue T, Li C, Yang DJ, Higuchi T, Oriuchi N, Yu D, Milas L, Hunter N, Wallace S, Kim EE, Podoloff DA (1999) Evaluation of In-111 DTPA-paclitaxel scintigraphy to predict response of murine tumors to paclitaxel. Ann Nucl Med 13:169
13. Kallinowski F, Schaefer C, Tyler G, Vaupel P (1989) In vivo targets of recombinant human tumour necrosis factor-alpha: blood flow, oxygen consumption and growth of isotransplanted rat tumours. Br J Cancer 60:555
14. Kerbel RS (2001) Clinical trials of antiangiogenic drugs: opportunities, problems, and assessment of initial results. J Clin Oncol 19:45S
15. Kestell P, Zhao L, Baguley BC, Palmer BD, Muller G, Paxton JW, Ching LM (2000) Modulation of the pharmacokinetics of the antitumour agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in mice by thalidomide. Cancer Chemother Pharmacol 46:135
16. Kuh HJ, Jang SH, Wientjes MG, Weaver JR, Au JL (1999) Determinants of paclitaxel penetration and accumulation in human solid tumor. J Pharmacol Exp Ther 290:871
17. Lash CJ, Li AE, Rutland M, Baguley BC, Zwi LJ, Wilson WR (1998) Enhancement of the anti-tumour effects of the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) by combination with 5-hydroxytryptamine and bioreductive drugs. Br J Cancer 78:439
18. Lew YS, Brown SL, Griffin RJ, Song CW, Kim JH (1999) Arsenic trioxide causes selective necrosis in solid murine tumors by vascular shutdown. Cancer Res 59:6033
19. Manthey CL, Qureshi N, Stutz PL, Vogel SN (1993) Lipopolysaccharide antagonists block taxol-induced signaling in murine macrophages. J Exp Med 178:695
20. Murata R, Overgaard J, Horsman MR (2001) Comparative effects of combretastatin A-4 disodium phosphate and 5,6-dimethylxanthenone-4-acetic acid on blood perfusion in a murine tumour and normal tissues. Int J Radiat Biol 77:195
21. Murata R, Overgaard J, Horsman MR (2001) Potentiation of the anti-tumour effect of hyperthermia by combining with the vascular targeting agent 5,6-dimethylxanthenone-4-acetic acid. Int J Hyperthermia 17:508
22. Murata R, Siemann DW, Overgaard J, Horsman MR (2001) Improved tumor response by combining radiation and the vascular-damaging drug 5,6-dimethylxanthenone-4-acetic acid. Radiat Res 156:503
23. Nicholson KM, Bibby MC, Phillips RM (1997) Influence of drug exposure parameters on the activity of paclitaxel in multicellular spheroids. Eur J Cancer 33:1291
24. Pedley RB, Boden JA, Boden R, Boxer GM, Flynn AA, Keep PA, Begent RH (1996) Ablation of colorectal xenografts with combined radioimmunotherapy and tumor blood flow-modifying agents. Cancer Res 56:3293
25. Pedley RB, Sharma SK, Boxer GM, Boden R, Stribbling SM, Davies L, Springer CJ, Begent RH (1999) Enhancement of antibody-directed enzyme prodrug therapy in colorectal xenografts by an antivascular agent. Cancer Res 59:3998
26. Pruijn FB, van Daalen M, Holford NH, Wilson WR (1997) Mechanisms of enhancement of the antitumour activity of melphalan by the tumour-blood-flow inhibitor 5,6-dimethylxanthenone-4-acetic acid. Cancer Chemother Pharmacol 39:541
27. Rewcastle GW, Kestell P, Baguley BC, Denny WA (1990) Light-induced breakdown of flavone acetic acid and xanthenone analogues in solution. J Natl Cancer Inst 82:528
28. Screnci D, Galettis P, Baguley BC, McKeage MJ (1998) Optimisation of an ICP-MS assay for the detection of trace levels of platinum in peripheral nerves. Atomic Spectroscopy 19:172
29. Screnci D, McKeage MJ, Galettis P, Hambley TW, Palmer BD, Baguley BC (2000) Relationships between hydrophobicity, reactivity, accumulation and peripheral nerve toxicity of a series of platinum drugs. Br J Cancer 82:966
30. Shimomura K, Manda T, Mukumoto S, Kobayashi K, Nakano K, Mori J (1988) Recombinant human tumor necrosis factor-alpha: thrombus formation is a cause of anti-tumor activity. Int J Cancer 41:243
31. Siemann DW, Mercer E, Lepler S, Rojiani AM (2002) Vascular targeting agents enhance chemotherapeutic agent activities in solid tumor therapy. Int J Cancer 99:1
32. Siim BG, Laux WT, Rutland MD, Palmer BN, Wilson WR (2000) Scintigraphic imaging of the hypoxia marker ^{99m}technetium-labeled 2,2′-(1,4-diaminobutane)bis(2-methyl-3-butanone) dioxime (^{99m}Tc-labeled HL-91; Prognox): noninvasive detection of tumor response to the antivascular agent 5,6-dimethylxanthenone-4-acetic acid. Cancer Res 60:4582
33. Silobrcic V, Suit HD (1967) Tumor-specific antigen(s) in a spontaneous mammary carcinoma of C3H mice. I. Quantitative cell transplants into mammary-tumor-agent-positive and -free mice. J Natl Cancer Inst 39:1113
34. Tannock IF (1998) Conventional cancer therapy: promise broken or promise delayed? Lancet 351 [Suppl 2]:9
35. Tannock IF, Lee CM, Tunggal JK, Cowan DS, Egorin MJ (2002) Limited penetration of anticancer drugs through tumor tissue: a potential cause of resistance of solid tumors to chemotherapy. Clin Cancer Res 8:878
36. Teicher BA, Holden SA, al Achi A, Herman TS (1990) Classification of antineoplastic treatments by their differential toxicity toward putative oxygenated and hypoxic tumor subpopulations in vivo in the FSaIIC murine fibrosarcoma. Cancer Res 50:3339
37. Vincent PW, Roberts BJ, Elliot WL, Leopold WR (1997) Chemotherapy with DMXAA (5,6-dimethylxanthenone-4-acetic acid) in combination with CI-1010 (1H-imidazole-1-ethanol,alpha-[[(2-bromoethyl)amino]methyl]-2-nitro-,mono hydrobromide (R isomer)) against advanced stage murine colon carcinoma 26. Oncol Rep 4:143
38. Wilson WR, Li AE, Cowan DS, Siim BG (1998) Enhancement of tumor radiation response by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid. Int J Radiat Oncol Biol Phys 42:905
39. Zhao L, Kestell P, Philpott M, Ching LM, Zhuang L, Baguley BC (2001) Effects of the serotonin receptor antagonist cyproheptadine on the activity and pharmacokinetics of 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Cancer Chemother Pharmacol 47:491
40. Zwi LJ, Baguley BC, Gavin JB, Wilson WR (1989) Blood flow failure as a major determinant in the antitumor action of flavone acetic acid. J Natl Cancer Inst 81:1005
41. Zwi LJ, Baguley BC, Gavin JB, Wilson WR (1994) Correlation between immune and vascular activities of xanthenone acetic acid antitumor agents. Oncol Res 6:79
42. Zwi LJ, Baguley BC, Gavin JB, Wilson WR (1994) The morphological effects of the anti-tumor agents flavone acetic acid and 5,6-dimethyl xanthenone acetic acid on the colon 38 mouse tumor. Pathology 26:161