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ORIGINAL ARTICLE

Antitumour action of 5,6-dimethylxanthenone-4-acetic acid in rats bearing chemically induced primary mammary tumours

Johnson J. Liu · Lai-Ming Ching · Michael Goldthorpe · Rachel Sutherland · Bruce C. Baguley · James A. Kirker · Mark J. McKeage

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Abstract
Purpose To evaluate the antitumour activity of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a vascular disrupting agent currently under phase II clinical trials in combination with cancer chemotherapy, in rats bearing chemically induced primary mammary tumours.
Methods Tumours were induced in female Wistar rats by injection of *N*-nitroso-*N*-methylurea at 100 mg/kg subcutaneously. A clinically relevant single dose of DMXAA (1,800 mg/m^2) was given to animals when tumours were measurable. Tumour volume, extent of necrosis and cytokine profiles were measured.
Results Compared with the control group, DMXAA treatment significantly delayed tumour doubling time and extended the time from treatment to euthanasia. Four of five DMXAA-treated animals showed necrosis involving 3.7–41.2% of the area of the tumour section at 24 h compared with none of four control animals ($P < 0.028$, Chi-square test). Intratumoural levels of TNFα, IL-6, VEGF and IL-1α were increased 4 h after DMXAA treatment.
Conclusions This study shows for the first time that DMXAA has significant in vivo antitumour activity against non-transplanted autochthonous tumours and in a host species other than the mouse.

Keywords DMXAA · Mammary carcinoma · Antiangiogenesis · TNFα · Cytokines

J. J. Liu · M. Goldthorpe · M. J. McKeage (✉)
Department of Pharmacology and Clinical Pharmacology,
Faculty of Medical and Health Sciences,
University of Auckland, Private bag 92019,
Auckland, New Zealand
e-mail: m.mckeage@auckland.ac.nz

L.-M. Ching · R. Sutherland · B. C. Baguley · J. A. Kirker
Auckland Cancer Society Research Centre,
Faculty of Medical and Health Sciences,
The University of Auckland, Auckland, New Zealand

Introduction

The therapeutic potential of antitumour agents that act by disrupting the blood supply of solid tumours is attracting a great deal of interest currently. These vascular disrupting agents primarily target established tumour blood vessels, in contrast to antiangiogenic agents, such as bevacizumab, which interfere with new vessel formation [1]. Solid tumours are dependent upon an established vasculature for provision of oxygen and nutrients and for their progression and metastasis. Tumour blood vessels differ from normal blood vessels, allowing the potential for selective therapeutic intervention [2, 3]. Two main classes of vascular disrupting agents are currently under clinical evaluation; the first is tubulin binders such as combretastatin A4 phosphate. The second class of agent exerts vascular disrupting effects via tubulin-independent mechanisms and is typified by 5,6-dimethylxanthenone-4-acetic acid (DMXAA).

The DMXAA acts by disrupting the tumour vasculature, directly and indirectly, causing apoptosis of tumour vascular endothelial cells and intratumoural induction of cytokines [4]. Non-clinical antitumour activity has been documented in a wide range of transplanted mouse tumour models as a single agent or in combination with cytotoxic chemotherapy [5], radiotherapy [6] and other therapeutic modalities [7].

However, little evidence of antitumour activity was seen in the 124 patients treated on single agent phase I clinical trials of DMXAA despite these patients tolerating much higher doses (MTD 4,500 mg/m^2) and higher plasma concentrations of DMXAA (C_{max} 1.7 mM) than the mouse (MTD 90 mg/m^2; C_{max} 0.6 mM) [8–12]. In addition, some of the effects of DMXAA, such as activation of NF-κB, have been reported to differ between human and murine cells [13]. We sought to gain some understanding of these interspecies differences in the pharmacology of DMXAA by studying the action of the drug in a third animal species, the rat.

Previous studies of DMXAA in the rat have established that the MTD for this species (1,800 mg/m^2) is similar to the clinical phase II dose of the drug (1,200–1,800 mg/m^2) but higher than the MTD for the mouse (90 mg/m^2). In addition, at their respective MTDs, peak plasma concentrations of DMXAA are similar in rats (2.2 mM) and humans (1.7 mM) but higher than the mouse (0.6 mM). In the studies we now report, use was made of an autochthonous tumour model where primary mammary tumours were induced by exposing menarcheal female rats to a chemical carcinogen (NMU). It is known that mammary tumours developing in female rats after chemical carcinogen exposure are usually adenocarcinomas, sensitive to hormonal manipulation and begin as intraductal proliferations of dysplastic cells before progressing to invasive cancer with a stromal reaction [14]. In these respects, these rodent tumours appear to reflect the biology of human primary breast cancer possibly more closely than serially passaged, transplanted rodent tumour models. In the current study, rats bearing measurable NMU-induced primary mammary tumours were treated with a single clinically relevant dose of DMXAA and tumour growth, necrosis and cytokine induction were measured.

Material and methods

Drugs and chemicals

The sodium salt of DMXAA was provided by the Auckland Cancer Society Research Centre, and it was dissolved in 5% sodium bicarbonate for administration to the animals. DMXAA solutions were made fresh for each experiment and protected from light because of its photosensitivity. *N*-nitroso-*N*-methylurea and other reagents were purchased from Sigma Chemical Co., St. Louis, USA, unless indicated otherwise.

Animals, carcinogen and DMXAA treatment

The study was approved by the Animal Ethics Committee at the University of Auckland. Forty-eight age-matched, female Wistar rats, 43 days old, were bred and housed in the Animal Resource Unit under constant temperature, humidity and 12 h darkness–light cycle with access to clean food and water ad libitum according to the institutional animal welfare guidelines.

The NMU was dissolved in acidified sterile water to an injection volume of 10 ml/kg body weight and administered to animals subcutaneously at a single dose of 100 mg/kg. Potential sites of tumour development were inspected twice per week post-NMU. Tumours were measured twice weekly using a vernier calliper and the tumour volume was calculated as $a^2b \times 0.52$, where a and b are the transverse and longitudinal tumour diameters, respectively. Animals with measurable tumours (at least one tumour of 1 cm in the longitudinal diameter) were randomly allocated to the treatment group, receiving a single i.p. dose of DMXAA at 300 mg/kg (1,800 mg/m^2), or to the control group, receiving drug vehicle. Animals were monitored daily for their general condition and weighed twice weekly with a 125-day observation period following treatment. Animals were immediately euthanized with an i.p. dose of sodium pentobarbitone (90 mg/kg, Chemstock Animal Health Ltd, Christchurch, New Zealand) at the end of the experiment or when they developed signs of poor health such as ruffled fur, hunched posture or decreased mobility, greater than 15% body weight loss or large (≥2.5 cm diameter), ulcerated or complicated tumours. The end points for determining the activity of DMXAA were defined as tumour regression (>50% reduction in tumour volume), the time from treatment to tumour volume doubling and tripling and the time from treatment to euthanasia.

Histopathological assessment

Histological tumour responses were investigated in a subgroup of animals. Twenty-four hours after treatment of animals with a single i.p. dose of DMXAA ($n = 5$) or vehicle ($n = 4$), tumours were removed, rinsed with saline and fixed in 4% formaldehyde for 24 h. The tissue blocks were cut into 3 mm thick slices, embedded in paraffin, sectioned at 6 μm and stained with Ehrlich's haematoxylin and eosin. Analysis of tumour sections was blinded to treatment allocation. Montage images of whole tumour sections were taken using an Eclipse TE-2000E inverted microscope fitted

with a digital camera system and controlled by NIS-Elements AR version 2.10 imaging software (Nikon, Kanagawa, Japan). The images were examined and the proportion of necrotic area was calculated as a percentage of the area of a tumour section, which was measured and expressed as pixel^2 using AxioVision 3.1 imaging process software (Carl Zeiss, Hallbergmoos, Germany).

TUNEL assay of tumour apoptosis

Tumours were collected 4 h post-treatment with DMXAA ($n = 3$) or drug vehicle ($n = 3$), embedded in Tissue-Tek O.C.T. compound (Sakura Finetechnical Co., Ltd, Tokyo, Japan) and snap-frozen in liquid nitrogen. Cryosections of 10 μm thickness were collected on poly-L-lysine-coated slides and stored at −80°C. TUNEL assay was performed to identify double-stranded DNA breaks using an In Situ Cell Death Detection Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA strand breaks were visualised under a Leica DMR fluorescence microscope (Leica, Bensheim, Germany) at a detection wavelength of 520 nm. Images of 10 randomly selected fields per sample, which were not located in necrotic regions, were taken by an affiliated digital camera (Nikon, Tokyo, Japan). The total number of apoptotic bodies with positive fluorescence staining in DMXAA- or vehicle-treated tumours was quantified using high-throughput Metamorph image analysis software (v.6.2.6, Molecular Devices Co., Union city, CA, USA) and assays at the Department of Pharmacology High-Content Screening Laboratory at the University of Auckland (Auckland, New Zealand; http://www.health.auckland.ac.nz/pharmacology/discovery-1/).

Determination of cytokines

Four hours after treatment with a single dose of DMXAA at 300 mg/kg or drug vehicle, animals were euthanized using sodium pentobarbitone (i.p., 90 mg/kg). Blood samples of 5 ml each were collected and allowed to coagulate on ice overnight. Following centrifugation at 2,000 × g for 30 min at 4°C (Labofuge 400R, Heraeus Instruments, Hanau, Germany), serum was obtained and stored at −80°C. Dissected tumour tissue was homogenized using an electrical homogenizer (OMNI International, Inc., Warrenton, VA, USA) in pre-cooled PBS containing 1% protease inhibitor cocktail (Sigma), 1 ml PBS per gram of tumour tissue. The resulting supernatants collected following two centrifugations (3,000 and 14,000 × g, 30 min at 4°C) were used for further analysis. TNFα in serum and tumour tissue ($n = 10$) was determined using a commercially available ELISA kit (OptEIA rat Kit, PharMingen, San Diego, CA, USA) following the manufacturer's instructions. Other cytokines/chemokines in tumour tissue ($n = 6$) were measured using a Luminex 100 v.1.7 instrument controlled by Masterplex software and a Linco-Plex rat 24-plex kit (Linco Research, St. Charles, MO, USA) following the manufacturer's instructions.

Statistical analysis

The survival analysis with different endpoints, two-tailed t test, Chi-square test, log-rank test and other graphic representation were processed using Prism 3.0 software (GraphPad Software, San Diego, CA, USA), SigmaPlot 9.01 (Systat Software, Inc., Point Richmond, CA, USA) or SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA). A P value of <0.05 was considered significant.

Results

NMU tumour induction

About 90% of animals (44 of 48) developed measurable tumours after NMU treatment, mostly in the cervical-thoracic and abdominal–inguinal mammary glands, with 29% of the animals developing a second primary mammary tumour. The average time from NMU treatment to the development of measurable tumours was 95 ± 31 days. Twenty-five tumour-bearing animals were used for a growth-delay experiment. Twenty of these tumours were examined histopathologically at the end of the growth-delay experiment; 17 (85%) were mammary adenocarcinoma (Fig. 2a), 2 (10%) were squamous carcinoma and 1 (5%) was a sarcoma. An additional 9 tumour-bearing animals were used for histological assessment of tumour necrosis 24 h post-treatment and 10 animals for measuring tumour cytokine profiles 4 h post-treatment. NMU treatment was well tolerated except for the late development of lens cataracts in 5/48 (10%) animals.

DMXAA treatment

Animals were treated with a single dose of DMXAA (300 mg/kg i.p.) or vehicle when tumours became measurable. DMXAA was well tolerated except for transient clinical signs such as lethargy and ruffled fur, and the animals recovered completely within 2 h of treatment.

Variability was observed in the starting tumour volumes and subsequent tumour growth among the animals within each treatment group (Table 1). However, compared with the control group, administration of a single dose of DMXAA delayed tumour growth (Fig. 1a, b), increased the time to tumour doubling (Fig. 1c) and increased time from treatment to euthanasia (Fig. 1d). The differences in tumour growth and time to euthanasia between the DMXAA- and vehicle-treated groups were statistically significant (Table 2). The median time from treatment to doubling of tumour volume was 10 days in the control group and 44 days in the DMXAA group (hazard ratio 2.23, 95% CI 1.02–6.80; log-rank, $P < 0.05$). The median time from treatment to tripling of tumour volume was 32 days in the control group and 59 days in the DMXAA group (hazard ratio 2.42, 95% CI 1.02–8.49; log-rank, $P < 0.05$). The median time from treatment to euthanasia was 26 days in the control group and 69 days in the DMXAA group (hazard ratio 2.55, 95% CI 1.16–7.89; log-rank, $P < 0.05$). Of 10 DMXAA-treated animals, 4 showed greater than 50% reduction in tumour volume compared with none of 15 control animals ($P < 0.008$, Chi-square test).

The extent of necrosis in tumours was assessed histologically in a subset of animals at 24 h after treatment (Fig. 2a–d). H&E-stained sections of tumours from vehicle-treated animals ($n = 4$) showed no necrosis but

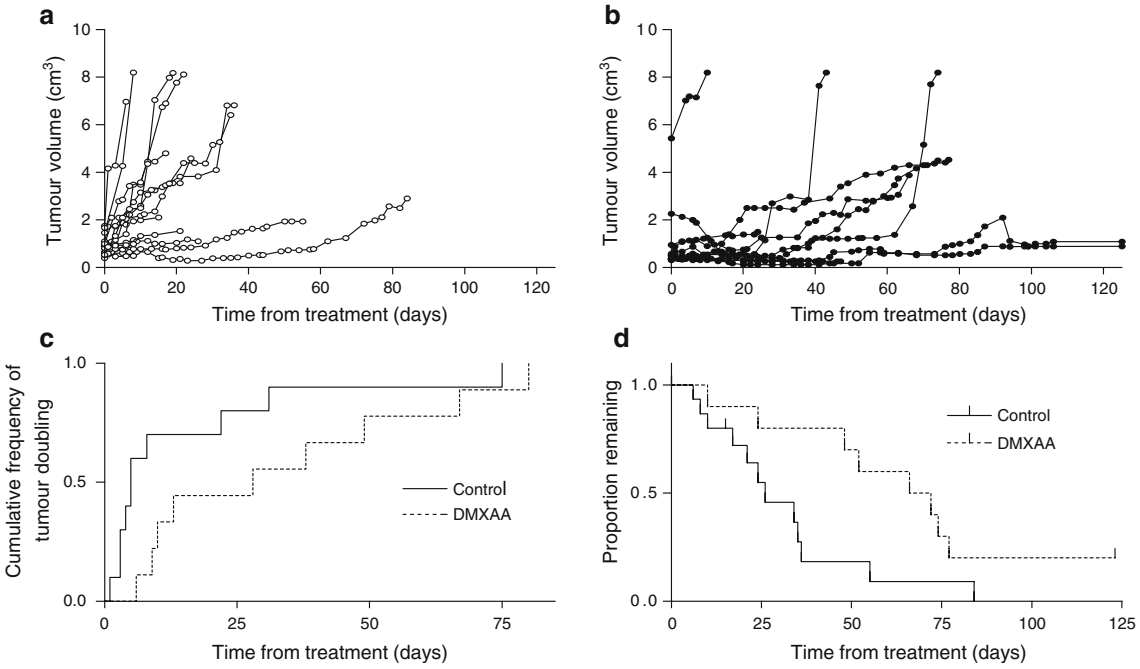

Fig. 1 Tumour growth curves in animals following treatment with drug vehicle (**a**) or DMXAA (**b**). Cumulative frequency of tumour volume doubling (**c**) and time to euthanasia of animals (**d**) (log-rank, $P < 0.05$) from the time of treatment with DMXAA (*broken line*) or vehicle (*solid line*)

Table 1 Comparison of tumour size, time to euthanasia, tumour necrosis and TUNEL index between DMXAA- and vehicle-treated animals bearing NMU-induced mammary tumours

Treatment	Tumour volume (cm^3)[a]		Time from treatment to euthanasia (days)	Tumour necrosis at 24 h (% of tumour area, $n = 5$)	TUNEL index at 4 h ($n = 3$)[b]
	Before treatment	At euthanasia			
Control	0.39–1.75 (0.82)[c]	0.5–8.2 (3.2)[c]	5–84 (21)[c]	0	107.0 ± 8.8
DMXAA	0.31–5.43 (0.62)	0.3–8.2 (3.55)	10–106 (69)	16.7 ± 16.4[b, d]	234.4 ± 45.9[e]

[a] Tumour volume was calculated with the equation: $0.52a^2b$, where a and b were the transverse and longitudinal diameters of tumour, respectively

[b] Data values are expressed as mean ± standard deviation

[c] Data values are expressed as range with median in parenthesis

[d] $P < 0.05$ versus control using Chi-square test

[e] $P < 0.05$ versus control using two-tailed t test

Table 2 Statistical analysis of the time from treatment to doubling and tripling of tumour volume and time from treatment to euthanasia of DMXAA- and vehicle-treated animals bearing NMU-induced mammary tumours

Endpoint	Median time (days)			Hazard ratio (95% CI)	Log-rank test (*P* value)
	Control ($n = 15$)	DMXAA ($n = 10$)	Ratio (95% CI)		
Tumour doubling	10	44	0.23 (−0.19 to 0.65)	2.23 (1.02–6.80)	0.045
Tumour tripling	32	59	0.54 (0.16 to 0.92)	2.42 (1.02–8.49)	0.046
Euthanasia	26	69	0.38 (−0.03 to 0.79)	2.55 (1.16–7.89)	0.024

occasional foci of apoptotic cells in the centre of tumour lobules (Fig. 2a, b). However, multiple areas of necrosis (demarcated in Fig. 2c) involving 3.7, 16.8, 21.7 and 41.2% (mean ± SD 16.7 ± 16.4%, Table 1) of the total area of the tumour sections, respectively, were observed in four of five DMXAA-treated animals ($P < 0.05$ versus control, Chi-square test). In addition to areas of necrosis, the number and size of the apoptotic

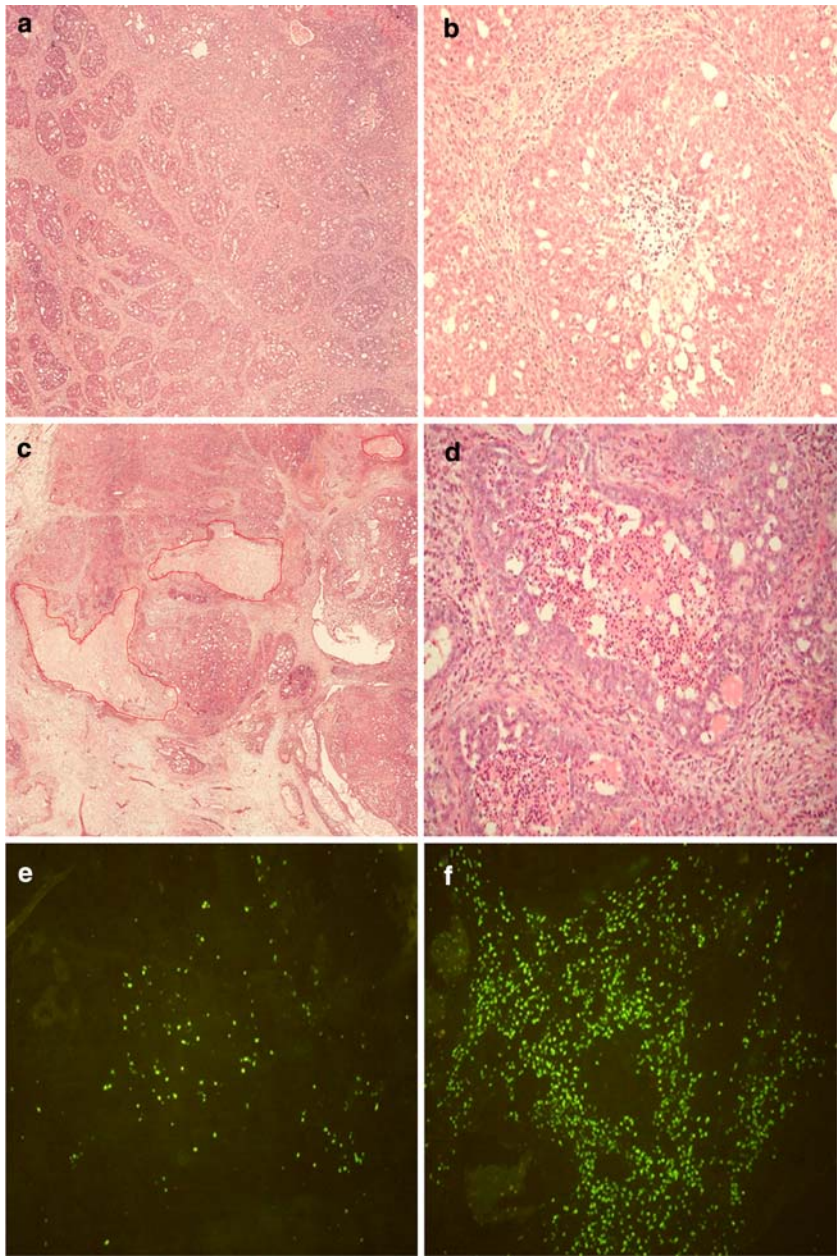

Fig. 2 Representative micrographs of NMU-induced mammary tumours from animals 24 h (H&E staining; **a**–**d**) or 4 h (TUNEL staining; **e** and **f**) after treatment with the drug vehicle (**a**, **b** and **e**) or a single dose of DMXAA at 300 mg/kg (**c**, **d** and **f**). Extensive necrotic areas (outlined in **c**, ×40) and enlarged foci of apoptotic cells in the central area of lobule (**d**, ×100) of tumours were shown in DMXAA-treated animals in comparison with vehicle-treated control (**a**, ×40; **b**, ×100). TUNEL staining (**e** and **f**, ×100) showed the increase in the number of positive apoptotic bodies with DMXAA treatment

foci in the centre of tumour lobules were increased in the DMXAA-treated animals (Fig. 2d). Moreover, as shown in Table 1 and Fig. 2 (e, f), TUNEL staining revealed a significant increase in the total number of apoptotic body with positive fluorescence staining per image in DMXAA-treated tumours compared with the control (234.4 ± 45.9 vs. 107.0 ± 8.8, $P < 0.05$).

Tumour cytokine induction

ELISA was used to measure TNFα in tumour tissue and serum at 4 h after DMXAA or vehicle treatment. As shown in Fig. 3, intratumoural TNFα content was significantly elevated in DMXAA-treated animals, by 3.5-fold, compared with the control animals (417.7 ± 190.3 vs. 119.9 ± 17.7 pg/g, $P < 0.05$). However, no significant difference in serum TNFα level was found between the two groups (34.9 ± 9.5 vs. 25.4 ± 0.3 pg/ml, $P > 0.05$).

A Luminex multiplex immunoassay was used to determine tumour cytokine profiles. A total of 24 cytokines/chemokines were measured in tumours collected from animals 4 h after treatment with DMXAA or vehicle. None of the 24 tumour cytokines decreased after DMXAA treatment. Data for nine cytokines that apparently increased in tumours after DMXAA treatment are shown in Fig. 4. DMXAA induced MIP-1α, IL-6, VEGF, KC, IL-1α, IP-10, RANTES, MCP-1 and IL-1β in rat mammary tumours. For IL-6, VEGF and IL-1α, the differences in the mean tumour cytokine levels between the DMXAA- and control groups reached statistical significance. In addition, all the cytokines shown in Fig. 4 were elevated in at least one of three DMXAA-treated animals to levels greater than the upper 95% confidence interval of the control group.

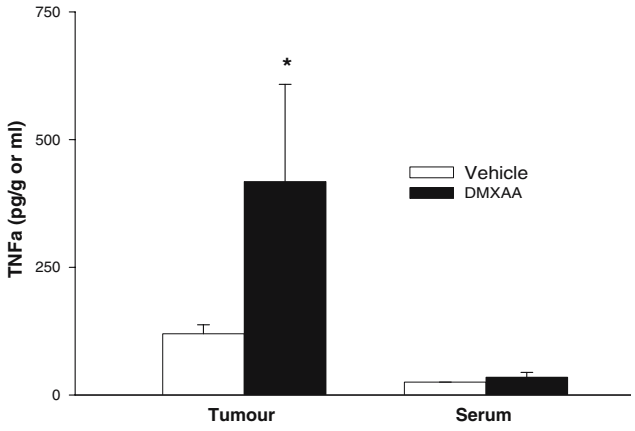

Fig. 3 TNFα induction in tumour and serum at 4 h after treatment. Animals bearing NMU-induced mammary tumours were treated with a single dose of DMXAA at 300 mg/kg or vehicle. *Horizontal bars* represent the standard errors. *Asterisk* indicates a significant level of difference (*t* test, $P < 0.05$) between DMXAA- ($n = 3$) and vehicle-treated animals ($n = 7$)

Discussion

In this study, we have demonstrated for the first time that DMXAA has significant antitumour activity in rats bearing chemically induced primary mammary tumours. A single dose of DMXAA was shown to significantly delay the growth of NMU-induced primary mammary tumours in rats and to extend the time from treatment to euthanasia of tumour-bearing animals in comparison to a control group. Median tumour doubling time, median tumour tripling time and median time from treatment to euthanasia were increased by DMXAA by approximately 4.4-, 1.8- and 2.7-fold, respectively. The differences between the control and DMXAA groups in these parameters of tumour growth and time to euthanasia were statistically significant.

With respect to the pharmacology of DMXAA, the rat may more closely reflect the human situation than the mouse. The dose of DMXAA that achieved therapeutic activity in the current study of tumour-bearing rats was 300 mg/kg or 1,800 mg/m^2. This dose is similar to the doses of DMXAA currently undergoing clinical evaluation in phase II trials that range from 1,200 to 1,800 mg/m^2 [12]. In contrast, clinically relevant doses cannot be readily achieved in the mouse because of toxicity limiting the maximum tolerated dose to about 90 mg/m^2 in this species [9]. In addition, at their respective MTDs, peak plasma concentrations of DMXAA are similar in humans (1.7 mM) [8, 12] and rats (2.2 mM), but higher than in the mouse (0.6 mM) [9]. Although it cannot be concluded from the results of this study that the rat is a better model than the mouse for studying DMXAA, the rat may have the advantage over the mouse of allowing investigation of clinically relevant doses and systemic exposures of DMXAA.

This study was also the first to show that DMXAA has significant activity in a primary autochthonous tumour model. Autochthonous tumours grow within the same host and at the same anatomical site at which the tumour has originated spontaneously or by induction. Although a direct comparison of autochthonous and transplanted rat tumours was not made in the current study, previous studies suggest that autochthonous and transplanted rodent tumours may differ in their vascular development, growth kinetics and responsiveness to vascular targeting agents [15–17].

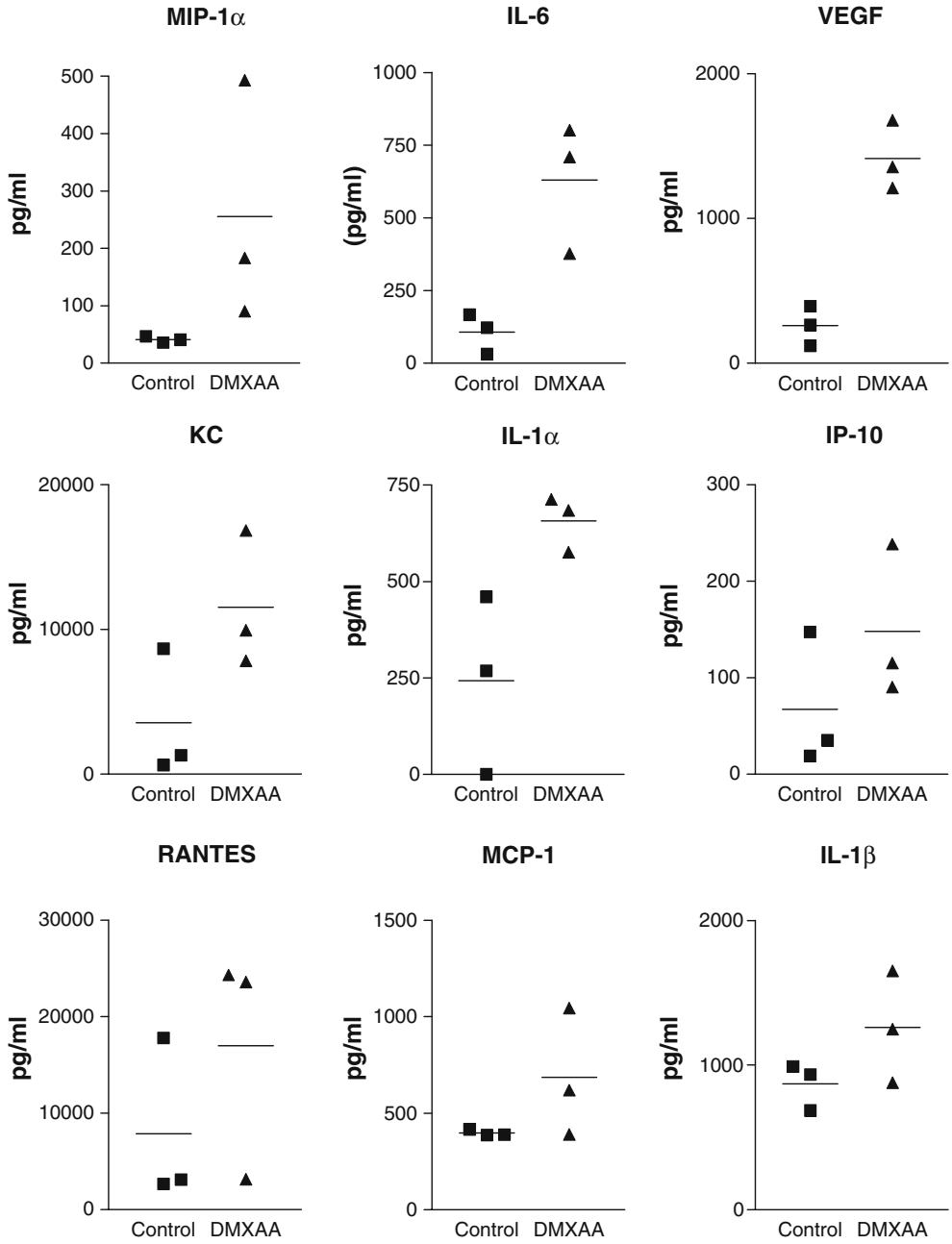

Fig. 4 Cytokine profiles in NMU-induced rat mammary tumours 4 h after treatment of animals with DMXAA at 300 mg/kg (*filled triangle*) or vehicle (*filled square*). *Horizontal bars* represent means ($n = 3$)

While it cannot be concluded that autochthonous tumour models are better than transplanted tumour models for the non-clinical testing of agents like DMXAA, autochthonous tumours may have the advantage of all their cellular components having originated from the same host.

The therapeutic effects of DMXAA observed in tumour-bearing rats may have resulted from the intratumoural induction of several cytokines and vascular damage. An assessment of rat tumour cytokine profiles and histology allowed some insight into the action of DMXAA, although only one time-point and dose level was examined. Within rat mammary tumours, DMXAA induced TNFα, IL-6, VEGF, IL-1α and other cytokines. Some of the cytokines induced by DMXAA with rat tumours are known to cause tumour vascular damage, haemorrhagic necrosis and altered vascular permeability [18–25]. Histological evaluation revealed evidence of necrosis occurring in tumours in DMXAA-treated animals, indicative of a

disrupted blood supply caused probably by complex effects on the vasculature either directly or indirectly by drug treatment. The extent of these histological changes, however, varied between tumours from different animals. Similar changes in intratumoural cytokine levels, and in tumour histology, have been previously reported in mice after DMXAA treatment [26–30]. However, unlike rats and humans, serum TNFα is induced in mice, which may contribute to the relative sensitivity of mice to the toxicity of DMXAA [26–28, 31].

In conclusion, a single clinically relevant dose of DMXAA delayed the growth of carcinogen-induced primary rat mammary tumours and extended the time from treatment to euthanasia of tumour-bearing animals. This therapeutic activity of DMXAA was accompanied by the intratumoural induction of multiple cytokines at 4 h and tumour necrosis at 24 h, after dosing. This is the first description of the antitumour action of DMXAA against non-transplanted autochthonous tumours and in a host species other than the mouse.

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References

1. Siemann DW, Bibby MC, Dark GG et al (2005) Differentiation and definition of vascular-targeted therapies. Clin Cancer Res 11:416–420
2. Kelland L (2005) Targeting established tumor vasculature: a novel approach to cancer treatment. Curr Cancer Ther Rev 1:1–9
3. Neri D, Bicknell R (2005) Tumour vascular targeting. Nat Rev 5:436–446
4. Baguley BC (2003) Antivascular therapy of cancer: DMXAA. Lancet Oncol 4:141–148
5. Siim BG, Lee AE, Shalal-Zwain S et al (2003) Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Cancer Chemother Pharmacol 51:43–52
6. Wilson WR, Li AE, Cowan DS et al (1998) Enhancement of tumor radiation response by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid. Int J Radiat Oncol Biol Phys 42:905–908
7. Baguley BC, Wilson WR (2002) Potential of DMXAA combination therapy for solid tumors. Expert Rev Anticancer Ther 2:593–603
8. Rustin G, Bradley C, Galbraith S et al (2003) 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), a novel antivascular agent: phase I clinical and pharmacokinetic study. Br J Cancer 88:1160–1167
9. Kestell P, Paxton JW, Rewcastle GW et al (1999) Plasma disposition, metabolism and excretion of the experimental antitumour agent 5,6-dimethylxanthenone-4-acetic acid in the mouse, rat and rabbit. Cancer Chemother Pharmacol 43:323–330
10. Jameson MB, Thompson PI, Baguley BC et al (2003) Clinical aspects of a phase I trial of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a novel antivascular agent. Br J Cancer 88:1844–1850
11. Zhou SF, Tingle MD, Kestell P et al (2002) Species differences in the metabolism of the antitumour agent 5,6-dimethylxanthenone-4-acetic acid in vitro: implications for prediction of metabolic interactions in vivo. Xenobiotica 32:87–107
12. McKeage MJ, Fong P, Jeffery M et al (2006) 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) in the treatment of refractory tumours: a phase I safety study of a vascular disrupting agent. Clin Cancer Res 12:1776–1784
13. Woon ST, Reddy CB, Drummond CJ et al (2005) A comparison of the ability of DMXAA and xanthenone analogues to activate NF-kB in murine and human cell lines. Oncol Res 15:351–364
14. Russo J, Russo IH (1996) Experimentally induced mammary tumors in rats. Breast Cancer Res Treat 39:7–20
15. Field S, Burney IA, Needham S et al (1991) Are transplanted tumours suitable as models for studies on vasculature? Int J Radiat Biol 60:255–260
16. Izumi Y, di Tomaso E, Hooper A et al (2003) Responses to antiangiogenesis treatment of spontaneous autochthonous tumors and their isografts. Cancer Res 63:747–751
17. Fenton BM, Lord EM, Paoni SF (2001) Intravascular HBO_2 saturations, perfusion and hypoxia in spontaneous and transplanted tumor models. Int J Cancer 93:693–698
18. Sgadari C, Angiolillo AL, Cherney BW et al (1996) Interferon-inducible protein-10 identified as a mediator of tumor necrosis in vivo. Proc Natl Acad Sci USA 93:13791–13796
19. Dinney CP, Bielenberg DR, Perrotte P et al (1998) Inhibition of basic fibroblast growth factor expression, angiogenesis, and growth of human bladder carcinoma in mice by systemic interferon-a administration. Cancer Res 58:808–814
20. Carswell EA, Old LJ, Kassel RL et al (1975) An endotoxin-induced serum factor that causes necrosis of tumours. Proc Natl Acad Sci USA 72:3366–3670
21. Watanabe N, Niitsu Y, Umeno H et al (1988) Toxic effect of tumor necrosis factor on tumor vasculature in mice. Cancer Res 48:2179–2183
22. Manda T, Shimomura K, Mukumoto S et al (1987) Recombinant human tumor necrosis factor-alpha: evidence of an indirect mode of antitumor activity. Cancer Res 47:3707–3711
23. Dvorak HF, Gresser I (1989) Microvascular injury in pathogenesis of interferon-induced necrosis of subcutaneous tumors in mice. J Natl Cancer Inst 81:497–502
24. Johnson CS, Chang MJ, Braunschweiger PG et al (1991) Acute haemorrhagic necrosis of tumours induced by interleukin-1-alpha—effects independent of tumor necrosis factor. J Natl Cancer Inst 83:842–848
25. Baban DF, Seymour LW (1998) Control of tumour vascular permeability. Adv Drug Deliv Rev 34:109–119
26. Joseph WR, Cao Z, Mountjoy KG et al (1999) Stimulation of tumors to synthesize tumor necrosis factor-alpha in situ using 5,6-dimethylxanthenone-4-acetic acid: a novel approach to cancer therapy. Cancer Res 59:633–638
27. Philpott M, Baguley BC, Ching LM (1995) Induction of tumour necrosis factor-a by single and repeated doses of the antitumour agent 5,6-dimethylxanthenone-4-acetic acid. Cancer Chemother Pharmacol 36:143–148
28. Ching LM, Goldsmith D, Joseph WR et al (1999) Induction of intratumoral tumor necrosis factor (TNF) synthesis and

hemorrhagic necrosis by 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in TNF knockout mice. Cancer Res 59:3304–3307

29. Pang JH, Cao Z, Joseph WR et al (1998) Antitumour activity of the novel immune modulator 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in mice lacking the interferon-gamma receptor. Eur J Cancer 34:1282–1289
30. Cao Z, Baguley BC, Ching LM (2001) Interferon-inducible protein 10 induction and inhibition of angiogenesis in vivo by the antitumor agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Cancer Res 61:1517–1521
31. Zhao L, Ching LM, Kestell P et al (2002) The antitumour activity of 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in TNF receptor-1 knockout mice. Br J Cancer 87:465–470