Cancer Chemother Pharmacol (1995) 36: 143–148

ORIGINAL ARTICLE

Martin Philpott · Bruce C. Baguley · Lai-Ming Ching

Induction of tumour necrosis factor-α by single and repeated doses of the antitumour agent 5,6-dimethylxanthenone-4-acetic acid

Received: 13 July 1994/Accepted: 13 October 1994

Abstract 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), a low-molecular-weight biological response modifier scheduled for clinical evaluation, induced synthesis of tumour necrosis factor-α (TNF-α) in serum of mice, with maximal activity being observed at 2–3 h after administration. At a dose of 27.5 mg/kg, DMXAA induced similar TNF-α concentrations as did flavone-8-acetic acid given at its maximum tolerated dose (MTD; 330 mg/kg), whereas 8-methylxanthenone-4-acetic acid, which has no antitumour activity, did not induce serum TNF-α at its MTD (440 mg/kg). The dependence of schedule on TNF-α induction was studied by giving DMXAA to mice in two doses of 27.5 mg/kg each separated by different intervals. An interval of 0 (i.e. 55 mg/kg given in a single dose) produced a TNF-α concentration 9-fold that produced by a single dose of 27.5 mg/kg. This dose, although higher than the MTD of 30 mg/kg, did not affect the health of mice at the time of assay (3 h). An interval of 1 day produced very low levels of serum TNF-α after the second injection. An interval of 3 days produced high levels of serum TNF-α after the second injection (9-fold that detected in mice receiving 27.5 mg/kg in a single dose) but no long-term toxicity, whereas an interval of 7 days produced an intermediate response. Thus, the first dose can either potentiate or suppress the TNF-α response to a second dose. Mice with advanced subcutaneous colon 38 tumours were treated either with a single dose of DMXAA (27.5 mg/kg) or with a divided dose (two doses of 27.5 mg/kg given 3 days apart). Both the cure rate and the tumour-growth delay were enhanced by the divided-dose schedule. The results are relevant to the design of clinical administration schedules of DMXAA and emphasise the importance of TNF-α induction in the antitumour response.

Key words Antitumor activity · Colon carcinoma · Schedule dependence

Introduction

The investigational antitumour agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA), developed in this laboratory [29], is scheduled for clinical evaluation as a more active and dose-potent analogue of flavone acetic acid (FAA), synthesised by Atassi et al. [2]. Both FAA and DMXAA show excellent activity against a range of murine transplantable tumours [25, 29], and a striking feature of these compounds is their ability to induce haemorrhagic necrosis of subcutaneous tumours [29, 31]. This effect is attributable to vascular collapse [11, 35]. FAA induces production of tumour necrosis factor-α (TNF-α) [19], and antibodies to TNF-α inhibit FAA-induced tumour vascular collapse [20] and partially inhibit its antitumour activity [26]. DMXAA also induces TNF-α production [10], suggesting that the ability of drugs of this type to induce TNF-α contributes to the antitumour activity.

Other biological effects of FAA and DMXAA in mice include the elevation of natural killer (NK) cells [3, 34] through the production of interferons [19], activation of macrophage tumouricidal activity [4], and the production of nitric oxide [32]. DMXAA stimulates higher levels of macrophage tumouricidal activity [8] and nitric oxide production [32] than does FAA. In mice it is 12-fold more dose-potent than FAA and induces a greater number of cures [29].

This study was supported by the Auckland Division of the Cancer Society of New Zealand and by the Health Research Council of New Zealand

M. Philpott · B.C. Baguley · L.-M. Ching (✉)
Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag 92079, Auckland, New Zealand.

FAA has failed to exhibit clinical activity as a single agent [18]. Analysis of peripheral blood samples collected from patients undergoing FAA therapy showed that FAA did not have the same degree of immunomodulatory activity in humans as it did in mice [13, 15]. It was suggested that FAA exhibited species specificity and that the lack of clinical activity was due to its lower efficiency in stimulating human host-cytotoxic mechanisms. The observation that FAA induces TNF-α in mouse but not human cells [13], together with the finding that DMXAA is much more active than FAA in stimulating human HL-60 cells in vitro to produce TNF-α mRNA [9], suggests that DMXAA may have greater potential as a clinical antitumour agent than does FAA.

We examined details of the induction of TNF-α by single- and repeated-dose schedules of DMXAA in the present study. We also examined the relationship between the antitumour activity of and TNF-α production by DMXAA. The results may be relevant to the forthcoming clinical trial of DMXAA.

Materials and methods

Materials

FAA was obtained through the courtesy of the National Cancer Institute (USA). DMXAA and 8-methylxanthenone-4-acetic acid (8-MeXAA) were synthesised in this laboratory [27, 29]. Solutions of FAA and DMXAA were prepared fresh for each experiment by dissolving the drugs in 5% sodium bicarbonate and were protected from light [28]. Rabbit anti-mouse TNF-α antibody (lot 101009) was purchased from Endogen Inc. (Boston, Mass, USA).

Mice and tumours

$(C_{57}Bl/6xDBA/2)F_1$ hybrid (BDF_1) mice, bred in the laboratory animal facilities, were used at ages ranging between 8 and 12 weeks. For serum TNF-α determinations, mice under anaesthesia were bled and the blood allowed to coagulate before centrifugation and collection of serum. Antitumour activity was measured using colon 38 adenocarcinoma, which was passaged in BDF_1 mice and implanted subcutaneously (1-mm^3 fragments) in the flanks of anaesthetised (sodium pentobarbital, 90 mg/kg) mice. Growth-delay determinations were initiated on day 10 after implantation, when the tumours were approximately 5 mm in size. Tumour-bearing mice were injected intraperitoneally with drug (0.1 ml/10 g body weight) and tumours were measured using calipers three times weekly thereafter. Tumour volumes were calculated as $0.52 \times a^2 \times b$, where *a* and *b* are the minor and major axes of the tumour. The arithmetic means were calculated for each time point (counting non-palpable tumours as zero volume) and the growth delay was determined as the number of days required for the treated tumours to reach a volume of 250 mm^3.

TNF-α bioassay

Serum TNF-α levels were measured in a standard cytotoxicity assay using actinomycin D-treated L929 cells [17]. L929 cells (3×10^4 cells/well) were cultured overnight in flat-bottomed microwells (Nunc, Kamstrup, Roskilde, Denmark). Actinomycin D (Merck, Sharpe and Dohme, Rahway, N.J., USA) was added to a final concentration of 8 μg/ml, serum samples to be assayed were added (100 μl/well) in triplicate or quadruplicate to the first row of cells in a total volume of 300 μl, and sequential 3-fold dilutions of the serum were then performed over the length of the microwell plates. In some experiments, anti-TNF-α antibody was added before incubation. The cultures were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2 in air. Cell killing was assessed using a colorimetric assay using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Serva, Heidelberg, Germany) as previously described [12]. MTT (500 μg/ml) was added to the cultures, which were incubated for another hour to allow dark blue crystals to appear. Culture supernatant was then removed and 100 μl dimethyl sulphoxide (Prolabo, Paris, France) was added to solubilise the crystals. Absorbance at 570 nm was measured using an automatic enzyme-linked immunosorbent assay (ELISA) reader (MR 600, Dynatech, Alexandria, Va, USA). Dose-response curves were constructed and the points above and below the dilution causing a 50% reduction in staining intensity were determined. A regression through this line was used to calculate both the TNF-α concentration and its standard error, whereby 1 unit of TNF-α was defined as the amount required to reduce specific staining by 50%.

Results

Serum TNF-α production in response to DMXAA

In initial experiments, we examined the TNF-α activity in serum collected at 30 min to 6 h after the injection of DMXAA at a dose (27.5 mg/kg) that has previously been shown to cause maximal immunostimulation. Activity, as detected by killing of L929 cells, was detected in sera collected at 2 and 3 h after drug treatment, with the 3-h sample giving twice the amount of TNF-α yielded by the sample collected after 2 h. No activity was detected at 30 min, 1 h or 4 h (Fig. 1). A dose-response curve was then determined using a 3-h blood collection time. TNF-α activity increased with dose up to the maximum tolerated dose (MTD) of 30 mg/kg DMXAA (Fig. 2). Doses above the MTD caused no sign of animal distress at the time the mice were killed for serum collection (3 h), except at the maximal dose tested (400 mg/kg), where slight diarrhoea, ruffled fur and apparent hypothermia were observed. Maximal levels of TNF-α were obtained at a DMXAA dose of 55 mg/kg, with lower production being observed at doses of up to 400 mg/kg (Fig. 2).

To confirm that DMXAA was producing TNF-α rather than another cytokine, L929 TNF-α assays were conducted in the presence of 1, 5 or 10 μg rabbit antibody specific against murine TNF-α. The response of the L929 cells was 47%, 62% and 83%, respectively, of that of cultures receiving no antibody.

Serum TNF-α production in response to FAA and 8-MeXAA

Maximal TNF-α production in response to FAA was detected at a dose of 330 mg/kg, which is also the MTD

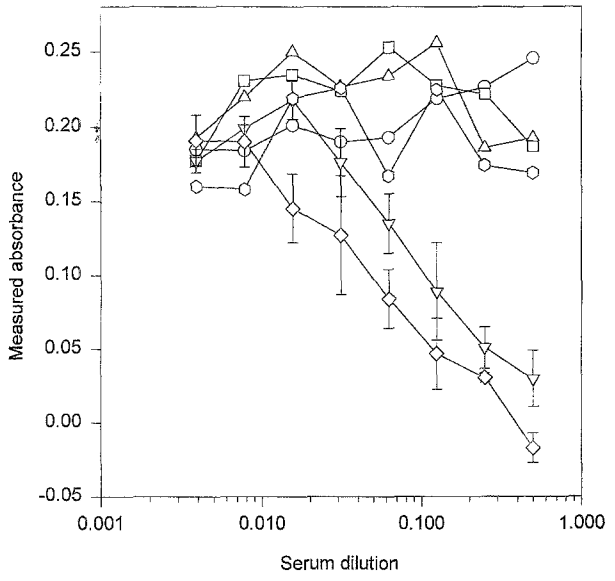

Fig. 1 Time course of TNF-α activity induced by DMXAA. Sera prepared from control mice (○) and from mice at 30 min (□), 1 h (△), 2 h (▽), 3 h (◇) or 4 h (*hexagons*) after injection with DMXAA (27.5 mg/kg) were assayed at the indicated dilutions for TNF-α activity using actinomycin D-sensitised L929 cells (note that in this experiment, cells were sensitised with only 1 μg actinomycin D/ml rather than with the optimal sensitising concentration of 8 μg/ml). *Points* represent mean absorbance (from triplicate wells) and *vertical bars* represent standard errors. For clarity, error bars are displayed only for the 2- and 3-h time points

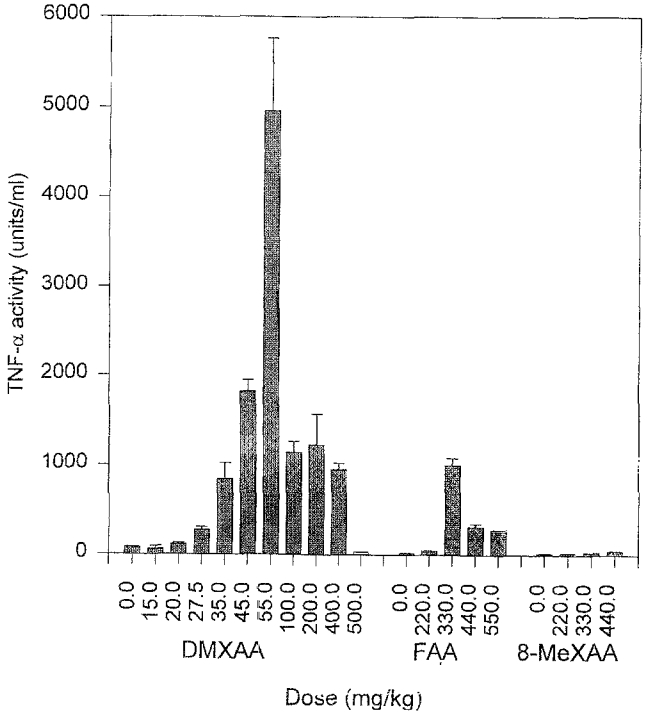

Fig. 2 Dose response of TNF-α induction by DMXAA, FAA and 8-MeXAA. Mice were treated with varying doses of each of the agents and the sera prepared 3 h later were assayed for TNF-α activity. Units of TNF-α were determined from the reciprocal of serum dilution that gave 50% killing of L929 cells. *Vertical bars* represent standard errors of determinations

for its antitumour activity (Fig. 2). The activity of TNF-α induced by FAA at 330 mg/kg was less than a quarter of that induced by DMXAA at 55 mg/kg. Mice receiving more than the MTD became lethargic, exhibiting diarrhoea and apparent hypothermia, and produced lower amounts of TNF-α. TNF-α production by 8-MeXAA, an analogue of DMXAA with no antitumour activity [27], was extremely low at all doses tested (Fig. 2). However, the same type of toxicity was manifest as observed for the active TNF-α inducers. All mice receiving 220 and 330 mg/kg 8-MeXAA exhibited diarrhoea and lethargy, and one of the three mice receiving 440 mg/kg 8-MeXAA died within 3h.

Effect of using split doses of DMXAA on TNF-α production

It was not feasible to measure the antitumour response to DMXAA at the dose required for maximal TNF-α production (55 mg/kg) since earlier work had indicated that this dose was lethal, with deaths occurring at 12–24 h after administration. We therefore examined the effect of giving DMXAA in two doses of 27.5 mg/kg spaced 1, 3 or 7 days apart. TNF-α levels were found to vary significantly depending on the interval between the two injections (Fig. 3). When the interval was 1 day, TNF-α was suppressed as compared with the group receiving one injection of 27.5 mg/kg. When the interval was 3 days, TNF-α activity was comparable with that observed following a single dose of 55 mg/kg and significantly higher ($P < 0.01$) than that seen following

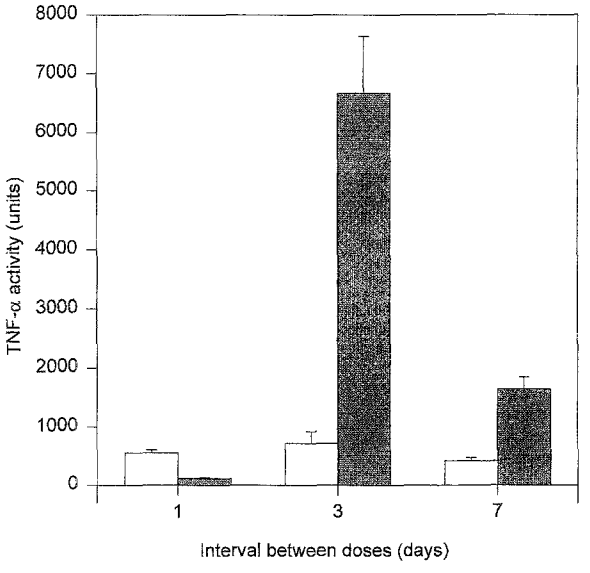

Fig. 3 TNF-α activity in response to two doses of DMXAA. Mice were given DMXAA either as a single dose (27.5 mg/kg; *unshaded bars*) or as a split dose (27.5 mg/kg each, separated by 1, 3 or 7 days; *shaded bars*). Sera were prepared 3 h after the final injection and assayed for TNF-α. *Vertical bars* represent standard errors of determinations

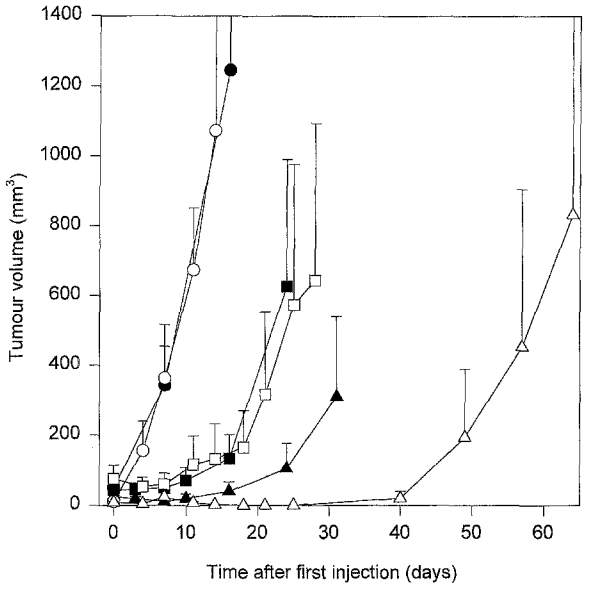

Fig. 4 Tumour growth following treatment with DMXAA. Two independent experiments were performed, the first using groups of 10 mice (*solid symbols*) and the second, groups of 5 mice (*open symbols*). Mice implanted 10 days earlier with colon 38 tumours received either no drug (*circles*), DMXAA as a single dose of 27.5 mg/kg (*squares*) or DMXAA as two doses of 27.5 mg/kg given 3 days apart (*triangles*). The *symbols* indicate mean tumour volumes and the *vertical bars* represent the standard errors of determinations, which were calculated for all mice in each group (including those with cures, accounting for the large bars at late time points after treatment)

a single dose of 27.5 mg/kg. When the interval was 7 days, TNF-α activity was reduced but nonetheless significantly higher than that noted following a single dose of 27.5 mg/kg. Moreover, mice receiving two injections of 27.5 mg/kg spaced 3 days apart survived over a long term, showing no deleterious effect despite the high level of TNF-α produced.

Effect of split doses of DMXAA on antitumour activity

We next tested the antitumour activity of DMXAA given in two doses of 27.5 mg/kg spaced 3 days apart. A single dose of DMXAA at 27.5 mg/kg gave 4/10 cures and a growth delay of 10 days (Fig. 4). Administration of a second dose 3 days later gave 8/10 cures and extended the growth delay to 24 days, with no drug toxicity being observed. A second independent experiment provided 2/5 cures and a growth delay of 20 days on the single-dose schedule and 4/5 cures and a growth delay of 40 days on the double-dose schedule (Fig. 4).

Discussion

The results described herein demonstrate the dose and schedule dependence of induction of TNF-α by DMXAA, a drug that is scheduled for clinical evaluation against human malignancies. DMXAA, more dose-potent and curative than FAA against the murine colon 38 tumour [29], induces higher amounts of TNF-α activity than does FAA, whereas 8-MeXAA, which has no antitumour activity, does not induce TNF-α. Experiments with anti-TNF-α antibody confirmed that the assay was measuring TNF-α. Correlations between antitumour activity, elevation of NK cell activity [5], induction of nitric oxide production [32], and stimulation of macrophage tumouricidal activity [7] have previously been demonstrated for derivatives of xanthenone-4-acetic acid, suggesting that all are part of the same pleiotropic response. The role of each of these immune functions, including that of TNF-α, in the overall antitumour response is not clear.

The acute toxicity of DMXAA and FAA does not appear to be related to the degree of induced TNF-α activity. Lethargy, ruffled fur and hypothermia are observed within 1 or 2 h of drug treatment, before maximal TNF-α activity is obtained. Furthermore, 8-MeXAA, which is not a TNF-α inducer (Fig. 2), produces similar toxicity, and the dose that induces toxicity within 3 h are similar for DMXAA, FAA and 8-MeXAA. Antitumour activity was obtained with DMXAA at a dose 20-fold lower than that at which such acute toxicity become apparent. In this respect, DMXAA would appear to have a much improved therapeutic index over FAA, but this is masked by DMXAA-induced delayed toxicity which previous data have shown to result in deaths at between 12 and 24 h.

The haematological effects induced by DMXAA [6] are similar to those described for TNF-α or IL-1 [6, 22] and contrast with those induced by conventional direct cytotoxic agents [30]. Mice receiving purified TNF-α develop many of the symptoms of lipopolysaccharide-induced toxic shock syndrome, and it has been suggested that TNF-α singly can mediate this multitude of symptoms [33]. Later studies have suggested a more complex situation involving a cascade of cytokines, since agonists to IL-1 [23] or interferon-gamma (IFN-γ) [16] have also been shown to protect against endotoxic shock.

Mice given 55 mg/kg DMXAA in two divided doses spaced 3 days apart produce as much TNF-α after the second dose as do mice given 55 mg/kg in a single injection. Whereas a single dose of 55 mg/kg is known to be toxic within 24 h, mice treated on a divided-dose schedule do not suffer any apparent side effect despite having similar serum TNF-α activity. However, the timing between doses may be critical, since injections given 1 day apart result in suppression of TNF-α activity. A possible explanation for the decrease in activity observed when the second dose is given after only 1 day is that following activation, macrophages enter a refractory period during which they will not respond to a subsequent stimulus because of the induction of mitochondrial superoxide dismutase [1, 24]. The

mechanism for the enhanced activity observed when the doses are spaced 3 days apart is not clear but may reflect the stimulation by DMXAA of the synthesis of several other cytokines, including IFN-γ [14] and granulocyte/macrophage colony-stimulating factor (GM-CSF; Ching, unpublished data). IFN-γ can prime macrophages to give a heightened response to a subsequent stimulus, whereas GM-CSF can hasten the maturation and production of macrophages from their early stem-cell precursors [21]. We have previously demonstrated a 3-fold increase in the bone marrow haemopoietic precursor stem cells at 2 days following administration of DMXAA, consistent with the release of GM-CSF [6]. The enhanced response may thus result from increased numbers of macrophages, as well as from increased priming, as a result of cytokine release. The enhancement would be expected to be relatively short-lived, consistent with a reduced effect observed after 7 days (Fig. 3).

In summary, the present study shows DMXAA to be a good inducer of TNF-α in vivo. TNF-α production appears to correlate with antitumour activity, suggesting that TNF-α could be a useful indicator of biological response in individual patients during clinical trials. The double-dose administration schedule provides a higher cure rate and longer growth delays for colon 38 tumours than can be obtained using a single dose of DMXAA at the MTD (Fig. 4), strongly suggesting that the therapeutic index of DMXAA might be improved by using multiple-dose schedules.

References

1. Alexander HR, Doherty GM, Block MI, Kragel PJ, Jensen JC, Langstein HN, Walker E, Norton JA (1991) Single-dose tumor necrosis factor protection against endotoxin-induced shock and tissue injury in rats. Infect Immun 59: 3889–3894
2. Atassi G, Briet P, Berthelon J-J, Collonges F (1985) Synthesis and antitumor activity of some 8-substituted 4-oxo-4H-1-benzopyrans. Eur J Med Chem 5: 393–402
3. Ching L-M, Baguley BC (1987) Induction of natural killer cell activity by the antitumour compound flavone acetic acid (NSC 347512). Eur J Cancer Clin Oncol 23: 1047–1050
4. Ching L-M, Baguley BC (1988) Enhancement of in vitro toxicity of mouse peritoneal exudate cells by flavone acetic acid (NSC 347512). Eur J Cancer Clin Oncol 24: 1521–1525
5. Ching L-M, Joseph WR, Zhuang L, Atwell GJ, Rewcastle GR, Denny WA, Baguley BC (1991) Induction of natural killer activity by xanthenone analogues of flavone acetic acid: relation with antitumour activity. Eur J Cancer 27: 79–83
6. Ching L-M, McKeage M, Joseph WR, Kestell P, Zwi LJ, Baguley BC (1991) Haematological effects in mice of the antitumour agents xanthenone-4-acetic acid, 5,6-dimethylxanthenone-4-acetic acid and flavone acetic acid. Cancer Chemother Pharmacol 28: 414–419
7. Ching L-M, Finlay GJ, Joseph WR, Baguley BC (1991) In vitro methods for screening agents with an indirect mechanism of antitumour activity: xanthenone analogues of flavone acetic acid. Eur J Cancer 27: 1684–1689
8. Ching L-M, Joseph WP, Baguley BC (1992) Stimulation of macrophage tumoricidal activity by 5,6-dimethylxanthenone-4-acetic acid, a potent analogue of the antitumor agent flavone-8-acetic acid. Biochem Pharmacol 44: 192–195
9. Ching LM, Joseph WR, Crosier KE, Baguley BC (1994) Induction of tumor necrosis factor-alpha messenger RNA in human and murine cells by the flavone acetic acid analogue 5,6-dimethylxanthenone-4-acetic acid (NSC 640488). Cancer Res 54: 870–872
10. Ching L-M, Joseph WR, Zhuang L, Baguley BC (1994) Interaction between endotoxin and the antitumour agent 5,6-dimethylxanthenone-4-acetic acid in the induction of tumour necrosis factor and haemorrhagic necrosis of colon 38 tumours. Cancer Chemother Pharmacol (in press)
11. Evelhoch JL, Bissery M-C, Chabot GG, Simpson NE, McCoy CL, Heilbrun LK, Corbett TH (1988) Flavone acetic acid (NSC 347512)-induced modulation of murine tumor physiology monitored by in vivo nuclear magnetic resonance spectroscopy. Cancer Res 48: 4749–4755
12. Finlay GJ, Wilson WR, Baguley BC (1986) Comparison of in vitro activity of cytotoxic drugs toward human carcinoma and leukaemia cell lines. Eur J Cancer Clin Oncol 22: 655–662
13. Futami H, Eader LA, Komschlies KL, Bull R, Gruys ME, Ortaldo JR, Young HA, Wiltrout RH (1991) Flavone acetic acid directly induces expression of cytokine genes in mouse splenic leukocytes but not in human peripheral blood leukocytes. Cancer Res 51: 6596–6602
14. Futami H, Eader LA, Back TT, Gruys E, Young HA, Wiltrout RH, Baguley BC (1992) Cytokine induction and therapeutic synergy with interleukin-2 against murine renal and colon cancers by xanthenone-4-acetic acid derivatives. J Immunother 12: 247–255
15. Haworth C, O'Reilly SM, Chu E, Rustin GJS, Feldmann M (1993) Flavone acetic acid (FAA) with recombinant interleukin-2 (rIL-2) in advanced malignant melanoma. III. Cytokine studies. Br J Cancer 67: 1346–1350
16. Heinzel FP (1990) The role of IFN-gamma in the pathology of experimental endotoxemia. J Immunol 145: 2920–2924
17. Hogan MM, Vogel SN (1990) Measurement of tumor necrosis factor α and β. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W (eds) Current protocols in immunology, vol 1. Greene Publishing Associates/Wiley Interscience, New York, p 6.10.1
18. Kerr DJ, Kaye SB (1989) Flavone acetic acid — preclinical and clinical activity. Eur J Cancer Clin Oncol 25: 1271–1272
19. Mace KF, Hornung RL, Wiltrout RH, Young HA (1990) Correlation between in vivo induction of cytokine gene expression by flavone acetic acid and strict dose dependency and therapeutic efficacy against murine renal cancer. Cancer Res 50: 1742–1747
20. Mahadevan V, Malik STA, Meager A, Fiers W, Lewis GP, Hart IR (1990) Role of tumor necrosis factor in flavone acetic acid-induced tumor vasculature shutdown. Cancer Res 50: 5537–5542
21. Metcalf D (1991) Control of granulocytes and macrophages — molecular, cellular, and clinical aspects. Science 254: 529–533
22. Morrisey PJ, Charrier K, Alpert A (1985) In vivo administration of IL-1 induces thymic hypoplasia and increased levels of serum corticosterone. J Immunol 141: 1456–1463
23. Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC (1990) Interleukin-1 receptor antagonist reduces mortality from endotoxic shock. Nature 348: 550–552
24. Okamoto T, Watanabe N, Yamauchi N, Tsuji Y, Tsuji N, Itoh Y, Neda H, Niitsu Y (1992) Endogenous tumor necrosis factor exerts its protective function intracellularly against the cytotoxicity of exogenous tumor necrosis factor. Cancer Res 52: 5278–5281
25. Plowman J, Naryanan VL, Dykes D, Szarvasi E, Briet P, Yoder OC, Pauli KD (1986) Flavone acetic acid: a novel agent with preclinical antitumor activity against colon adenocarcinoma 38 in mice. Cancer Treat Rep 70: 631–638

26. Pratesi G, Rodolfo M, Rovetta G, Parmiani G (1990) Role of T cells and tumour necrosis factor in antitumour activity and toxicity of flavone acetic acid. Eur J Cancer 26: 1079–1083
27. Rewcastle GW, Atwell GJ, Baguley BC, Calveley SB, Denny WA (1989) Potential antitumor agents. 58. Synthesis and structure-activity relationships of substituted xanthenone-4-acetic acids active against the colon 38 tumor in vivo. J Med Chem 32: 793–799
28. 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: 528–529
29. Rewcastle GW, Atwell GJ, Zhuang L, Baguley BC, Denny WA (1991) Potential antitumor agents. 61. Structure-activity relationships for *in vivo* colon-38 activity among disubstituted 9-oxo-9H-xanthene-4-acetic acids. J Med Chem 34: 217–222
30. Selby P, Hobbs S, Viner C, Jackson E, Jones E, Newell D, Calvert AH, McElwain T, Fearon K, Humphreys J, Shiga T (1987) Tumour necrosis in man: clinical and biological observations. Br J Cancer 56: 803–808
31. Smith GP, Calveley SB, Smith MJ, Baguley BC (1987) Flavone acetic acid (NSC 347512) induces haemorrhagic necrosis of mouse colon 26 and 38 tumours. Eur J Cancer Clin Oncol 23: 1209–1212
32. Thomsen LL, Ching LM, Zhuang L, Gavin JB, Baguley BC (1991) Tumor-dependent increased plasma nitrate concentrations as an indication of the antitumor effect of flavone-8-acetic acid and analogues in mice. Cancer Res 51: 77–81
33. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ, Zentella A, Albert JD, Shires GT, Cerami A (1986) Shock and tissue injury induced by recombinant human cachectin. Science 234: 470–474
34. Wiltrout RH, Boyd MR, Back TC, Salup RR, Arthur JA, Hornung RL (1988) Flavone-8-acetic acid augments systemic natural killer cell activity and synergizes with IL-2 for treatment of murine renal cancer. J Immunol 140: 3261–3265
35. Zwi LJ, Baguley BC, Gavin JB, Wilson WR (1989) Blood flow failure as a major determinant in the antitumor action of flavone acetic acid (NSC 347512). J Natl Cancer Inst 81: 1005–1013