Improving Local Tumor Control by Combining Vascular Targeting Drugs, Mild Hyperthermia and Radiation

Michael R. Horsman, Rumi Murata and Jens Overgaard

From the Danish Cancer Society, Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus C, Denmark

Correspondence to: Michael R. Horsman, Danish Cancer Society, Department of Experimental Clinical Oncology, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark. Tel: +45 89 49 26 22. Fax: +45 86 19 71 09. E-mail: mike@oncology.dk

Acta Oncologica Vol. 40, No. 4, pp. 497–503, 2001

Improvement in local control in a foot-implanted (200 mm^3) C3H mouse mammary carcinoma by combining vascular targeting drugs, mild hyperthermia and radiation was investigated. The vascular targeting drug was flavone acetic acid (FAA; 150 mg/kg) intraperitoneally injected either 3 h before local tumor water-bath heating or 1 h after local tumor irradiation. For untreated tumors, the average (±1 S.E.) tumor growth time (TGT; time to reach 5× treatment volume) was 7.1 days (±0.4). This was increased to 9.2 days (±0.7) by using FAA. Heating also increased TGT, the effect being temperature and time dependent, and this heat response was further increased by FAA. The radiation dose (±95% confidence interval) to control 50% of tumors (TCD50) 90 days after irradiation was 52 Gy (50–55) for radiation alone. This was decreased to 42 Gy (39–45) by FAA, 47 Gy (45–50) by heating (41.5°C; 60 min) 4 h after irradiation, and to 28 Gy (22–35) by combining FAA and heat. Thus, vascular targeting drugs can improve the efficacy of mild hyperthermia and radiation.

Received 25 July 2000
Accepted 24 January 2001

It is well established that hyperthermia is an effective method for enhancing the radiation response of animal tumors in vivo (1, 2). However, despite numerous clinical studies with radiation and heat, only a few trials have actually demonstrated any clear benefit of this combination (3–5). One of the major reasons for this lack of success in the clinic is the failure to adequately heat human tumors. Most animal studies have shown that when radiation and hyperthermia are given simultaneously, then all heat temperatures will enhance radiation damage, the effect simply getting larger as the temperature increases (2). However, if the radiation and heat are given in a sequential schedule, as occurs in most clinical studies, then temperatures in excess of 42.5°C are typically required to substantially enhance tumor response to radiation, with little effect seen at the lower, mild hyperthermia temperatures (2). These lower thermal doses are the most commonly seen clinically (6). To improve this situation, one must either develop more efficient ways to heat human tumors or find a method that can turn the mild hyperthermia temperature into an effective treatment.

The blood flow to a solid tumor is probably the most important factor that influences the response of that tumor to hyperthermia. Blood flow is one of the major vehicles by which heat is dissipated, thus affecting the ability to heat the tumor (7, 8). It also controls the type of environment that exists within tumors (9, 10) and this will influence the degree of heat damage (11–13). The importance of tumor blood flow is clearly shown from studies in which the response of solid tumors to heat is significantly increased if tumors are heated when the blood flow has been compromised either by clamping (14–16) or drug manipulation (17–21).

One agent that is very effective in shutting down tumor blood flow is flavone acetic acid (FAA; (22–27)). This drug is a synthetic flavenoid that has been shown to have a broad spectrum of activity against murine solid tumors (28–30), primarily as a result of inducing vascular damage (31–33). Unfortunately, in preliminary clinical studies the drug was shown to be ineffective (34, 35). Nevertheless, because the drug has been extensively used in vivo it is the perfect agent for demonstrating 'proof-of-principle' effects of vascular damaging drugs. Indeed, by using FAA several studies have clearly shown the potential of vascular targeting drugs to enhance heat damage at high temperatures (26, 27, 36). What has never been demonstrated is whether

 ISSN 0284-186X

vascular targeting drugs can also influence the effect of heat at the more clinically realistic mild heat temperatures. Nor has it been shown if such drugs, with low temperature hyperthermia, can be used to improve the efficacy of radiation. This is important, because it is the most likely clinical application of such combination therapy. The aim of the current study was to use FAA to address these questions in a C3H mouse mammary carcinoma.

MATERIAL AND METHODS

Animal and tumor model

All experiments were performed on female C3D2F1/Bom (C3H/Tif female × DBA/2 male) mice. The tumor model used was the C3H mouse mammary carcinoma, the derivation and maintenance of which has been described previously (37). Experimental tumors were produced following sterile dissection of large flank tumors. Macroscopically viable tumor tissue was minced with a pair of scissors, and 5–10 µl of this material injected into the foot of the right hind limb of 10–14-week-old experimental animals. Treatments were carried-out when the tumors had reached a volume of about 200 mm^3, which generally occurred within 3 weeks after challenge, and was determined by the formula: $D1 \times D2 \times D3 \times \pi/6$ (where the D values represent three orthogonal diameters). All the experiments were performed under nationally approved guidelines for animal welfare.

Drug preparation

FAA was supplied by Lyonnaise Industrielle Pharmaceutique (LIPHA, Lyon, France) and was freshly prepared before each experiment by dissolving in a 1% Na_2CO_3 solution. Drug injections were given intraperitoneally (i.p.) at a constant volume of 0.02 ml/g mouse body weight.

Hyperthermia experiments

All heat treatments were administered locally to the tumor. To achieve this, non-anesthetized mice were restrained in specially constructed lucite jigs. The tumor-bearing legs were then exposed and loosely attached to the jig with tape, without impairing the blood supply to the foot. Hyperthermia was delivered by immersing the leg into a circulating water bath (type TE 623, from Heto, Birkerød, Denmark) stabilized to ± 0.02°C of the adjusted temperature. The water bath was covered with a lucite plate, with holes allowing immersion of the foot approximately 1 cm below the water surface. Previous measurements of intratumoral temperature have shown stabilization within a few minutes to approximately 0.2°C below the water bath temperature (18). The temperature of the water bath was therefore adjusted to 0.2°C above the desired tumor temperature. All temperature measurements were calibrated against a certified precision mercury thermometer. The response of tumors to heat treatment was assessed using a regrowth delay assay. Basically, tumor volume was measured 5 times each week after treatment, as described earlier, and the tumor growth time (TGT; time to reach 5 × treatment volume) determined.

Radiation treatments

Irradiations were given using a conventional therapeutic x-ray machine (240 kV; 15 mA; 2-mm Al filter; 1.1 mm Cu half-value layer; dose rate of 2.3 Gy/min). Dosimetry was accomplished by using an integrating chamber. All treatments to tumor-bearing feet were given locally to the tumors of non-anesthetized mice, which were restrained as described for the hyperthermia studies. Tumors only were irradiated, the remainder of the animal being shielded by 1 cm of lead. To secure homogeneity of the radiation dose, the tumors were immersed in a water bath with about 5 cm of water between the x-ray source and the tumor. Tumor response to treatment was assessed using a local tumor control assay. This involved observing the mice on a weekly basis and recording the percentage of animals in each treatment group, showing local tumor control 90 days after treatment.

RESULTS

Examples of the growth characteristics of this C3H mammary carcinoma under different conditions are illustrated

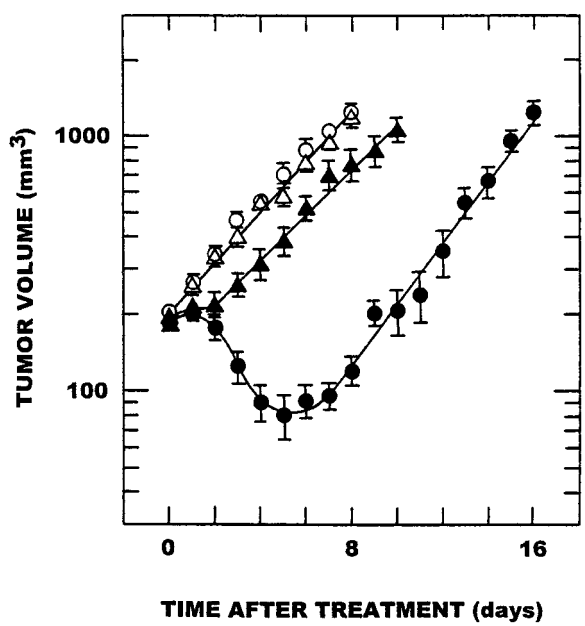

Fig. 1. Examples of the types of growth curves obtained with this C3H mammary carcinoma following different treatments. Mice with 200 mm^3 foot tumors were given either no treatment (△), i.p. injected with 150 mg/kg flavone acetic acid (FAA; ▲), local tumor heating at 40.5°C for 200 min (○), or injected with FAA 3 h prior to tumor heating (●), and tumor volume determined as a function of time after treatment. Results show means (± 1 S.E.) for an average of 14 mice/group.

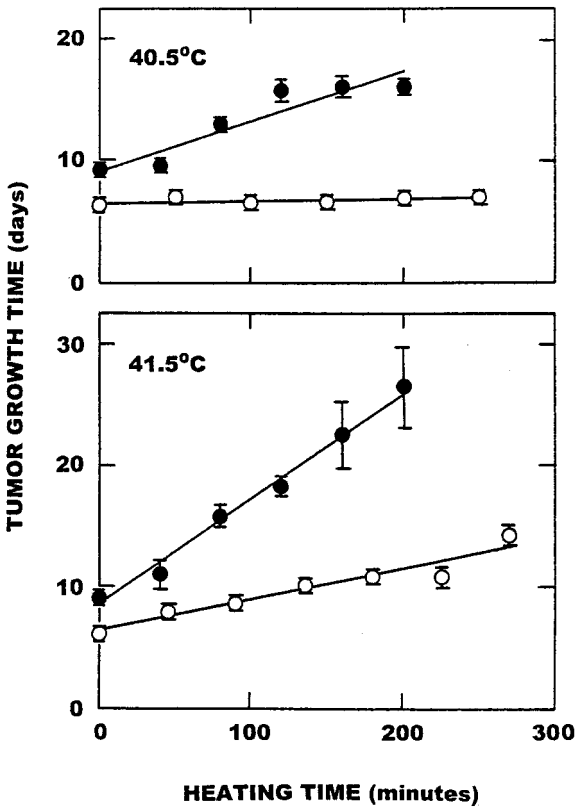

Fig. 2. The effect of flavone acetic acid (FAA; 150 mg/kg; i.p.) on the response of the C3H mammary carcinoma to heat. Mice were injected with FAA 3-h prior to local tumor heating at the indicated temperatures for various times and the tumor growth times (TGTs) determined. Results show means ($\pm$1 S.E.) for an average of 20 mice/group in animals given either heat alone (○) or FAA + heat (●). Lines were drawn following regression analysis using the individual TGTs for each mouse.

in Fig. 1. For untreated tumors, the growth was exponential with an average TGT ($\pm$1 S.E.) of 7.1 days ($\pm$0.4). Injecting mice with FAA (150 mg/kg; i.p.) when the tumors were around 200 mm^3 resulted in approximately a 2-day cessation in growth, after which time the tumors grew at the same rate as that in the control animals, giving rise to a TGT of 9.2 days ($\pm$0.7) and this was significantly (Student's t-test; $p < 0.05$) different from the TGT for control animals. Heating tumors at a temperature of 40.5°C, even for a period of 200 min, did not significantly inhibit tumor growth. However, if tumors were heated at 40.5°C 3 h after injecting FAA, there was a substantial increase in the heat response, with a TGT of 16.1 days ($\pm$0.7) being obtained, and this was significantly different from all the other treatment results shown in Fig. 1.

The TGT results for all heating times at 40.5°C, as well as those at 41.5°C, are shown in Fig. 2. For all the treatments illustrated in this figure, there is a linear relationship between the tumor response to heat and the time of heating. With 40.5°C alone, an almost flat response, with a slope of 0.002, was obtained. At 41.5°C the slope value was 0.027. In Fig. 2 the effect of FAA on the heat response curves is also shown. Apart from having an effect on tumor growth alone, the drug also enhanced the heat response at both temperatures and did so in a dose-modifying manner, reflected by the increase in the slope values to 0.040 and 0.087 at 40.5 and 41.5°C, respectively.

In Fig. 3, the slope values obtained from Fig. 2 are shown in an Arrhenius curve. This figure also contains slope values from previous results in this C3H mammary carcinoma for heat alone, and when combined with FAA at higher heat temperatures. For heat alone, there is the typical biphasic response with an inflection point around 42.5°C. If tumors are heated 3 h after injecting FAA, there is a clear increase in the slope value at each temperature. However, the degree of increase appears to be strongly dependent on the temperature at which tumors are heated, with a larger slope increase being seen at temperatures below 42.5°C.

Modification of the radiation response of this C3H mouse mammary carcinoma by a mild hyperthermia treatment of 41.5°C is illustrated in Fig. 4. Also shown in this figure is the effect of giving either FAA alone or when combined with the mild hyperthermia temperature. The radiation dose ($\pm$95% confidence interval) to control 50%

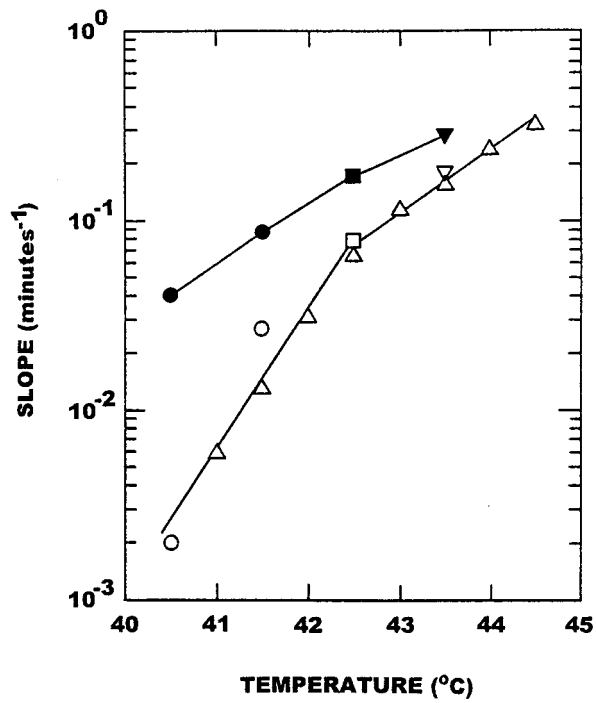

Fig. 3. Relationship between the temperature at which tumors were heated and the slopes of the tumor response curves taken from either the data shown in Fig. 2 (○ ●), from previously published results (△, 38; □ ■, 27), or from unpublished observations (▽ ▼). Results are for heat alone (open symbols), or flavone acetic acid (150 mg/kg) i.p. injected 3 h before heating (closed symbols).

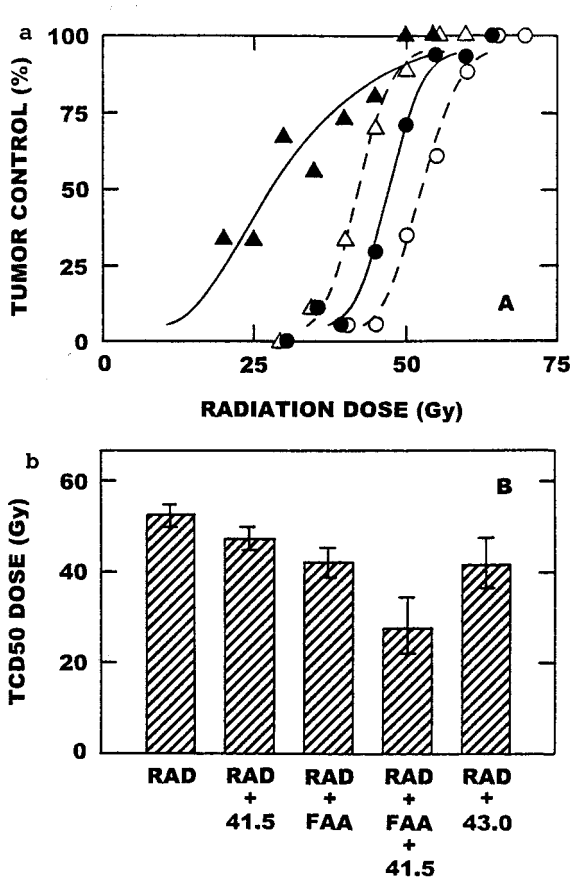

Fig. 4. The effect of flavone acetic acid (FAA; 150 mg/kg; i.p.) ± heat on the radiation response of the C3H mammary carcinoma. A. Tumors were locally irradiated with graded radiation doses and the percentage of animals in each treatment group showing local tumor control 90 days after treatment recorded. Mice were given either local tumor irradiation alone (○), locally heated for 60 min at 41.5°C starting 4 h after irradiation (●), injected with FAA 1 h after irradiation (△), or injected with FAA 1 h after irradiation and then heated at 41.5°C for 60-min 3 h later (▲). Points represent an average of 11 mice/group and all lines were fitted following logit analysis. B. The radiation (RAD) TCD50 values (±95% confidence limits) estimated from the data shown in Fig. 4A for all treatment groups, along with the TCD50 obtained for tumors that were irradiated and then 4 h later heated at 43°C for 60 min.

of tumors (TCD50) 90 days after irradiation was 52 Gy (50–55). Irradiating tumors and then 4 h later heating them at 41.5°C for 60 min resulted in a small, yet significant (χ^2 test; $p < 0.05$), decrease to a TCD50 value of 47 Gy (45–50). Injecting FAA (150 mg/kg; i.p.) 1 h after irradiation also significantly decreased the TCD50 value, to 42 Gy (39–45). By far, the greatest enhancement of radiation damage was seen when mice were injected with FAA 1-h after irradiation and the tumors were subsequently heated 3 h later at 41.5°C for 60 min. In that situation the TCD50 value was reduced to 28 Gy (22–35), and this was significantly different from the individual treatment results. Also shown in Fig. 4B is the TCD50 value, obtained when tumors were heated at 43°C for 60 min starting 4 h after giving radiation. With this more clinically relevant temperature, the TCD50 value was 41 Gy (37–47), and this was significantly greater than that found with the combination of FAA and the mild hyperthermia treatment.

DISCUSSION

Our study demonstrates the potential of vascular targeting drugs to enhance the anti-tumor activity of mild hyperthermia. Similar effects have previously been reported using FAA, but those studies involved using temperatures of 42.5°C and above (26, 27, 36). The enhancement of heat damage by FAA has been attributed to a drug-induced reduction in tumor blood flow (26, 27, 36). Indeed, in this study the selection of an FAA dose of 150 mg/kg and a 3-h time interval between drug injection and the start of heating was made simply because this was the optimal drug dose and time period for the maximal shutdown in blood perfusion in this C3H mouse mammary carcinoma (26, 27). Additional studies have also documented that the response of tumors to heat can be increased if blood flow is reduced by other methods, including clamping (14–16), using transient physiological modifiers such as 5-hydroxytryptamine and hydralazine (17, 18, 20, 21), and even a vascular damaging agent like tumor necrosis factor-α (TNF-α; 19). A reduced blood flow will lead to both a better tumor heating (18, 21, 39, 40) and an increase in tumor damage (18, 20, 21). This latter effect has been suggested to occur because the reduction in tumor blood flow results in an increase in tumor hypoxia and associated tumor acidosis, and several in vitro studies have shown that prolonged oxygen deprivation of cells will increase their sensitivity to heat, an effect which is due to hypoxia-induced metabolic pertubations rather than hypoxia per se (11–13).

One of the significant findings from our study was that the enhancement of heat damage by FAA was dependent on the heating temperature (Fig. 3). At 42.5°C and above, there was a relatively constant twofold increase in the slope of the heat-time response curve. Below 42.5°C, this enhancement increased as the temperature decreased. Our previous studies with FAA had shown that the maximum reduction in blood flow in this C3H mouse mammary carcinoma was almost identical to the maximal decrease seen in the same tumor model after treatment with the transient physiological modifier, hydralazine (18, 26, 27). Injecting mice with hydralazine and then heating tumors to 42.5°C and above, at the time of this maximal shutdown in blood flow, resulted in increases in the slopes of the heat-time response curves that were similar to those seen with FAA at the same temperatures. At 41.5°C the hydralazine-induced increase in slope was not significantly different from that seen with hydralazine at the higher

temperatures, but certainly less than that seen with FAA at this lower temperature. Why an increased response was seen with FAA at the milder heat temperatures is not known, but it may simply reflect the fact that FAA induces vascular damage, while agents like hydralazine do not.

Clinically, hyperthermia alone has no role to play in the curative treatment of tumors in humans (41). Its most likely clinical application is in combination with other cancer treatment modalities. Indeed, several studies have shown the benefit of combining hyperthermia with radiation to improve local control, especially in melanoma, breast and soft tissue sarcomas (3–5). Unfortunately, most studies show little benefit and this is most likely the result of a failure to heat tumors adequately to effective temperatures. While mild hyperthermia temperatures of around 41.5°C are easier to obtain clinically, results from preclinical studies suggest they are not very efficient at enhancing radiation damage and that temperatures of around 43°C are required (2). In our studies, we demonstrated that combining FAA with the more clinically achievable mild hyperthermia treatment of 41.5°C actually gave a tumor response that was just below that seen with the more clinically relevant temperature of 43°C alone (Fig. 3). This prompted us to investigate how these temperatures compared when combined with radiation. Injecting mice with FAA after irradiating the tumors actually improved local tumor control in this mouse tumor model, which supports the contention that vascular targeting agents that alone have a very modest effect on tumor growth (Fig. 1) may eventually have a significant role to play in improving radiation response in the clinic. More importantly for this study, we found that combining radiation, FAA and heat at 41.5°C for 60 min gave rise to a TCD50 value that was significantly lower than that seen for 43°C and radiation (Fig. 4). This suggests that if future clinical trials with hyperthermia and radiation were to include a vascular targeting drug in the regimen, it should be possible to overcome probably the most limiting factor influencing outcome, namely the failure to achieve adequate heating, and if so, then we should see many more trials showing benefit.

Despite a number of preclinical studies demonstrating a broad spectrum of activity for FAA in animal solid tumors (28–30), no demonstrable clinical activity was found in Phase II trials (34, 35). It has been suggested that the mechanism of action of FAA involves the production of TNF-α (42). An additional study has shown that while FAA could upregulate TNF-α mRNA in in vitro cultures of murine splenocytes, it had little effect in human HL-60 myelomonocytic leukemia cells (43), and it is this difference that is considered the reason for the discrepancy between animal and human results. But that has never been proven. What is known is that although the drug can induce a significant long-term shutdown in tumor perfusion, the effects on tumor growth (as seen in Fig. 1) are actually quite small (26, 27, 44). Such a small effect on tumor growth would never have been seen in any of the Phase II clinical trials. Similar minimal effects on tumor growth, despite substantial and sustained reductions in tumor perfusion, have also been observed with other vascular damaging agents, including colchicine, combretastatin A-4 disodium phosphate (CA4DP), 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and vinblastine (44–47) and this strongly supports the suggestion that for vascular targeting drugs to be effective, they must be combined with other more conventional therapies. It is interesting to speculate as to what might have been seen in the Phase II trials with FAA if the drug had been combined with additional treatments, especially hyperthermia and radiation.

However, rather than attempting to resurrect FAA, it may be more prudent to proceed with alternative treatments that target the tumor neo-vasculature. A number of drug, antibody- or gene therapy-based approaches are available (48). Two such drug-based agents are currently undergoing Phase I clinical testing. These are the new FAA derivative, DMXAA, and the tubulin-binding compound, CA4DP, which is a synthetic derivative of a compound originally isolated from the African Bush Willow *Combretum caffrum*. Both DMXAA and CA4DP have been documented to possess anti-tumor activity that involves a vascular component (46, 47, 49, 50). Furthermore, despite the minimal effects they have on tumor growth, both can significantly enhance radiation damage (51, 52). If these drugs successfully complete Phase I testing, and it can be demonstrated that they enhance the response to mild hyperthermia, with or without radiation, then they would clearly be the agents of choice for future clinical studies in this area.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Danish Cancer Society. The authors thank I. M. Johansen, D. Grand and M. Simonsen for excellent technical assistance.

REFERENCES

1. Field SB, Bleehen NM. Hyperthermia in the treatment of cancer. Cancer Treat Rev 1979; 6: 63–94.
2. Horsman MR, Overgaard J. Thermal radiosensitization in animal tumors: the potential for therapeutic gain. In: Urano M, Douple E, eds. Hyperthermia and oncology, vol. 2. Utrecht: VSP, 1989: 113–45.
3. Overgaard J, Gonzalez Gonzalez D, Hulshof MCCM, et al. Hyperthermia as an adjuvant to radiation therapy of recurrent or metastatic malignant melanoma. A multicentre randomized trial by the European Society for Hyperthermic Oncology. Int J Hyperthermia 1996; 12: 3–20.
4. Vernon CC, Hand JW, Field SB, et al. Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer results from five randomized controlled trials. Int J Radiat Oncol Biol Phys 1996; 35: 731–44.

5. Prosnitz LR, Maguire P, Anderson JM, et al. The treatment of high-grade soft tissue sarcomas with preoperative thermoradiotherapy. Int J Radiat Oncol Biol Phys 1999; 45: 941–9.
6. Oleson JR. Hyperthermia from the clinic to the laboratory: a hypothesis. Int J Hyperthermia 1995; 11: 315–22.
7. Jain RK, Grantham FH, Gullino PM. Blood flow and heat transfer in Walker 256 mammary carcinoma. J Natl Cancer Inst 1979; 62: 927–33.
8. Patterson J, Strang R. The role of blood flow in hyperthermia. Int J Radiat Oncol Biol Phys 1979; 5: 235–41.
9. Vaupel P. Oxygen supply to malignant tissues. In: Peterson HI, ed. Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors. Boca Raton: CRC Press Inc, 1979: 143–68.
10. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumor pH to the treatment of malignant disease. Radiother Oncol 1984; 2: 343–66.
11. Overgaard J, Bichel P. The influence of hypoxia and acidity on the hyperthermic response of malignant cells in vitro. Radiol 1977; 123: 511–4.
12. Gerweck LE, Nygaard TG, Burlett M. Response of cells to hyperthermia under acute and chronic hypoxic conditions. Cancer Res 1979; 39: 966–72.
13. Overgaard J, Nielsen OS. The role of tissue environmental factors on the kinetics and morphology of tumor cells exposed to hyperthermia. Ann New York Acad Sci 1980; 335: 254–80.
14. Suit HD. Hyperthermia in the treatment of tumors. In: Proc Int Symp cancer therapy by hyperthermia and radiation, Washington, 28–30 April. American College of Radiology, 1975: 107–14.
15. Hill SA, Denekamp J. The effect of vascular occlusion on the thermal sensitization of a mouse tumor. Br J Radiol 1978; 51: 997–1002.
16. Wallen CA, Colby TV, Stewart JR. Cell kill and tumor control after heat treatment with and without vascular occlusion in RIF-1 tumors. Radiat Res 1986; 106: 215–23.
17. Crile G. The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res 1963; 23: 372–80.
18. Horsman MR, Christensen KL, Overgaard J. Hydralazine-induced enhancement of hyperthermic damage in a C3H mammary carcinoma in vivo. Int J Hyperthermia 1989; 5: 123–36.
19. Kallinowski F, Moehle R, Vaupel P. Substantial enhancement of tumor hyperthermic response by tumor necrosis factor. In: Sugahara T, Saito M, eds. Hyperthermic oncology, vol. 1. London: Taylor and Francis, 1989: 258–9.
20. Kalmus J, Okunieff P, Vaupel P. Dose-dependent effects of hydralazine on microcirculatory function and hyperthermic response of murine FSaII tumors. Cancer Res 1990; 50: 15–9.
21. Honess DJ, Hu DE, Bleehen NM. A study of the mechanism of hydralazine enhancement of thermal damage in the KHT tumour. Int J Hyperthermia 1991; 7: 667–79.
22. Evelhoch JL, Bissery MC, Chabot GG, et al. Flavone acetic acid (NSC 347512)-induced modulation of murine tumor physiology monitored by in vivo nuclear magnetic resonance spectroscopy. Cancer Res 1988; 48: 4749–55.
23. Bibby MC, Double JA, Loadman PM, Duke CV. Reduction of tumor blood flow by flavone acetic acid: a possible component of therapy. J Natl Cancer Inst 1989; 81: 216–20.
24. Hill SA, Williams KB, Denekamp J. Vascular collapse after flavone acetic acid: a possible mechanism of its anti-tumour action. Eur J Cancer Clin Oncol 1989; 25: 1419–24.
25. Zwi LJ, Baguley BC, Gavin JB, Wilson WR. Blood flow as a major determinant in the antitumour action of flavone acetic acid. J Natl Cancer Inst 1989; 81: 1005–13.
26. Horsman MR, Chaplin DJ, Overgaard J. The effect of combining flavone acetic acid and hyperthermia on the growth of a C3H mammary carcinoma in vivo. Int J Radiat Biol 1991; 60: 385–8.
27. Horsman MR, Sampson LE, Chaplin DJ, Overgaard J. The in vivo interaction between flavone acetic acid and hyperthermia. Int J Hyperthermia 1996; 12: 779–89.
28. Corbett TH, Bissery MC, Wozniak A, et al. Activity of flavone acetic acid (NSC-347512) against solid tumors of mice. Invest New Drugs 1986; 4: 207–20.
29. Plowman J, Narayanan VL, Dykes D, et al. Flavone acetic acid: a novel agent with preclinical antitumor activity against colon adenocarcinoma 38 in mice. Cancer Treat Rep 1986; 70: 631–5.
30. Bibby MC, Double JA, Phillips RM, Loadman PM. Factors involved in the anti-cancer activity of the investigational agents LM985 (flavone acetic acid ester) and LM975 (flavone acetic acid). Br J Cancer 1987; 55: 159–63.
31. Smith GP, Calveley SB, Smith MJ, Baguley BC. Flavone acetic acid (NSC 347512) induced haemorrhagic necrosis of mouse colon 26 and 28 tumours. Eur J Cancer Clin Oncol 1987; 23: 1209–11.
32. Finlay GJ, Smith GP, Fray LM, Baguley BC. Effect of flavone acetic acid on Lewis lung carcinoma: evidence for an indirect effect. J Natl Cancer Inst 1988; 80: 241–5.
33. Zwi LJ, Baguley BC, Gavin JB, Wilson WR. The use of vascularized spheroids to investigate the action of flavone acetic acid on tumour blood vessels. Br J Cancer 1990; 62: 231–7.
34. Kerr DJ, Maughan T, Newlands E, et al. Phase II trials of flavone acetic acid in advanced malignant melanoma and colorectal carcinoma. Br J Cancer 1989; 60: 104–6.
35. Kaye SB, Clavel M, Dodion P, et al. Phase II trials with flavone acetic acid (NCS-347512, LM975) in patients with advanced carcinoma of the breast, colon, head and neck and melanoma. Invest New Drugs (Suppl) 1990; 8 (1): S95–9.
36. Sakaguchi Y, Maehara Y, Baba H, Kusumoto T, Sugimachi K, Newman RA. Flavone acetic acid increases the antitumor effect of hyperthermia in mice. Cancer Res 1992; 52: 3306–9.
37. Overgaard J. Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue in vivo. Int J Radiat Oncol Biol Phys 1980; 6: 1507–17.
38. Lindegaard JC, Overgaard J. Factors of importance for the development of the step-down heating effect in a C3H mammary carcinoma in vivo. Int J Hyperthermia 1987; 3: 79–91.
39. Voorhees WD, Babbs CF. Hydralazine-enhanced selective heating of transmissible venereal tumor implants in dogs. Eur J Cancer Clin Oncol 1982; 19: 1027–33.
40. Dewhirst MW, Prescott DM, Clegg S, et al. The use of hydralazine to manipulate tumour temperatures during hyperthermia. Int J Hyperthermia 1990; 6: 971–83.
41. Overgaard J. Rationale and problems in the design of clinical trials. In: Overgaard J, ed. Hyperthermic Oncology 1984, vol. 2. London: Taylor and Francis, 1985: 325–38.
42. Mahadevan V, Malik STA, Meager A, Fiers W, Lewis GP, Hart IR. Role of tumor necrosis factor in flavone acetic acid-induced tumor vasculature shutdown. Cancer Res 1990; 50: 5537–42.
43. Ching L-M, Joseph WR, Crosier KE, Baguley BC. Induction of tumor necrosis factor-α messenger RNA in human and murine cells by the flavone acetic acid analogue 5,6-dimethylxanthenone-4-acetic acid. (NSC 540488). Cancer Res 1994; 54: 870–2.
44. Hill SA, Sampson LE, Chaplin DJ. Anti-vascular approaches to solid tumour therapy: evaluation of vinblastine and flavone acetic acid. Int J Cancer 1995; 63: 119–23.

45. Baguley BC, Holdaway KM, Thomsen LL, Zhuang L, Zwi LJ. Inhibition of growth of colon 38 adenocarcinoma by vinblastine and colchicine: evidence for a vascular mechanism. Eur J Cancer 1991; 27: 482–7.
46. Lash CJ, Li AE, Rutland M, Baguley BC, Zwi LJ, Wilson WR. Enhancement of the anti-tumour effects of the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) by the combination with 5-hydroxytryptamine and bioreductive drugs. Br J Cancer 1998; 78: 439–45.
47. Chaplin DJ, Pettit GR, Hill SA. Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. Anticancer Res 1999; 19: 189–96.
48. Chaplin DJ, Dougherty GJ. Tumour vasculature as a target for cancer therapy. Br J Cancer 1999; 80 (Suppl) (1): 57–64.
49. Laws AL, Matthew AM, Double JA, Bibby MC. Preclinical in vitro and in vivo activity of 5,6-dimethylxanthenone-4-acetic acid. Br J Cancer 1995; 71: 1204–9.
50. Dark GD, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ. Combretastatin A-4, an agent that displays potent and selective toxicity towards tumor vasculature. Cancer Res 1997; 57: 1829–34.
51. Li L, Rojiani A, Siemann DW. Targeting the tumor vasculature with combretastatin A-4 disodium phosphate: effects on radiation therapy. Int J Radiat Oncol Biol Phys 1998; 42: 899–903.
52. Wilson WW, Li AE, Cowan D, Siim BG. Enhancement of tumor radiation response by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid. Int J Radiat Oncol Biol Phys 1998; 42: 905–8.