

Early Effects of Combretastatin-A4 Disodium Phosphate on Tumor Perfusion and Interstitial Fluid Pressure^{1,2}

Carsten D. Ley*, Michael R. Horsman[†] and Paul E. G. Kristjansen*

*Laboratory of Experimental Oncology, Institute of Molecular Pathology, University of Copenhagen, Frederik V's vej 11, DK-2100 Copenhagen, Denmark; [†]Department of Experimental Oncology, Aarhus University Hospital, Noerrebrogade 44, DK-8000 Aarhus C, Denmark

Abstract

Combretastatin-A4 disodium phosphate (CA4DP) is a vascular-disruptive agent that causes an abrupt decrease in tumor blood flow. The direct actions of CA4DP include increases in vascular permeability and destabilization of the endothelial cytoskeleton, which are thought to contribute to occlusion of the tumor vasculature. It has been proposed that increased permeability causes a transient increase in interstitial fluid pressure (IFP), which in turn could collapse intratumoral blood vessels. We examined the immediate effects of CA4DP on tumor IFP in C3H mammary carcinoma. Mice were treated with 100 mg/kg CA4DP by intraperitoneal injection. Tumor perfusion was recorded by laser Doppler flowmetry at separate time points, and IFP was recorded continuously by the wick-in-needle method. In this study, we found that CA4DP treatment resulted in a rapid reduction in tumor perfusion, followed by a decrease in IFP; no increases in IFP were observed. This suggests that CA4DP-induced reductions in tumor perfusion are not dependent on increases in IFP.

Neoplasia (2007) 9, 108–112

Keywords: Combretastatin-A4 disodium phosphate, interstitial fluid pressure, perfusion, wick-in-needle, vascular-damaging agent.

vascular shutdown and the limited bioavailability of the drug *in vivo*, the effects cannot be attributed to cytostatic or cytotoxic effects [12].

Treatment with CA4DP disrupts the tubulin cytoskeleton of dividing ECs within a few minutes [13,14]. Many different approaches have been employed to investigate how tubulin depolymerization causes an abrupt shutdown of tumor vasculature. Numerous factors are thought to play a role in this effect of CA4DP, including activation of the clotting system, changes in red blood cell deformability and rouleaux formation, adherence of leukocytes to the endothelium, and vascular constriction [15–17]. However, these mechanisms are not sufficient to explain abrupt decreases in tumor perfusion, as CA4DP causes only moderate vasoconstriction and retains an effect on tumors perfused with solutions free of cells and clotting factors [8,15,18,19].

The most important mechanisms are thought to involve EC shape changes and increased vascular permeability [12,18–20]. Several papers have suggested the principal mechanism of vascular occlusion involving vascular collapse caused by increases in interstitial fluid pressure (IFP) [12,15,18,20]. However, none of these studies includes actual measurements of IFP after CA4DP treatment. The only direct measurement of tumor IFP presently known to the authors was performed ≥ 1 hour after CA4DP treatment [Kristjansen et al. [4]³; Nielsen T, Murata R, Ley CD, Maxwell RJ, Kristjansen PEG, Stodkilde-Jorgensen H, Overgaard J, Horsman MR. Factors of importance for the vascular effects of combretastatin

Introduction

Combretastatins are potent inhibitors of microtubule assembly and are derivatives of the antineoplastic constituent isolated from the plant *Combretum cafrum* [1]. These agents cause tumor blood flow disruption and subsequent tumor cell death [2]. In particular, the water-soluble combretastatin-A4 prodrug, combretastatin-A4 disodium phosphate (CA4DP), has shown great clinical promise [3,4] and is now under evaluation in phase II clinical trials [5].

In numerous studies, combretastatin-A4 has been shown to have cytostatic and cytotoxic effects on proliferating tumor and endothelial cells (ECs) [6–10]. However, the ability of CA4DP to cause abrupt vascular shutdown *in vivo* at relatively nontoxic doses in a variety of tumors [11] is much more remarkable. Due to the rapid onset of

Abbreviations: CA4DP, combretastatin-A4 disodium phosphate; EC, endothelial cell; IFP, interstitial fluid pressure; LDF, laser Doppler flowmetry; MVP, microvascular pressure; PU, perfusion units

Address all correspondence to: Carsten D. Ley, MSc, The Edwin L. Steele Laboratory, Massachusetts General Hospital/Harvard Medical School, 100 Blossom Street, Boston, MA 02114. E-mail: science@ley.dk

¹This research was supported by grants from the Danish Cancer Society and the Danish Medical Research Council.

²Conflict-of-interest statements: Carsten D. Ley: none; Michael R. Horsman: serves as consultant for Oxigene, Inc., and assists with research programs determining certain relationships between Oxigene's drugs and radiation; Paul E. G. Kristjansen: none.

³A single dose of 250 mg/kg CA4DP was found to cause a significant decrease in IFP at 3 hours after treatment in C3H mouse mammary carcinoma (14.2 ± 1.3 mmHg compared to a control level of 27.2 ± 2.6 mmHg; $P = .003$).

Received 15 November 2006; Revised 8 January 2007; Accepted 8 January 2007.

Copyright © 2007 Neoplasia Press, Inc. All rights reserved 1522-8002/07/\$25.00
DOI 10.1593/neo.06733

A4 disodium phosphate (in preparation)⁴; Eikesdal et al. [22]), which is later than the onset of effects such as hypoxia [23], perfusion [24], and increased vascular resistance [18].

In this study, we investigate the time-dependent effects of CA4DP on IFP and tumor perfusion with the aim of clarifying the mode of action of combretastatins. C3H mammary carcinoma was chosen as a model system because of the well-characterized effects of CA4DP on this model: treatment with CA4DP specifically reduces tumor perfusion at doses well below the maximum tolerated dose, without causing similar effects in normal tissues [25,26]. In addition, this model has been shown to be amenable to accurate measurements of the acute changes in tumor perfusion elicited by vascular-targeting agents [24,27].

Materials and Methods

Animals and Tumors

A C3H mammary carcinoma grown subcutaneously in the right rear foot of 10- to 14-week-old female CDF1 mice was used in this study. The derivation and maintenance of this tumor have been described previously [28]. Tumor volume was calculated from the formula: $V_{\text{tumor}} = \pi/6[(d_1 - 0.5)(d_2 - 0.5)]^{3/2}$, where d_1 and d_2 values represent two orthogonal diameters [mm], and 0.5 mm is subtracted from each measured diameter to compensate for skin thickness. Experiments were performed when tumors had reached a size of approximately 250 to 350 mm³ (typically 3 weeks after inoculation). C3H tumors of this size contain only little necrosis [25], are uniformly firm without ulcerations, and permit normal use of the foot, but are big enough to accommodate the needle for IFP measurements. The mice were kept in standard housing conditions with free access to food and water; lighting was controlled in a 12-hour light/dark cycle. National and institutional guidelines for animal welfare and experimental conduct were followed.

Drug Preparation

CA4DP was supplied by Oxigene, Inc. (Watertown, MA). CA4DP was dissolved in isotonic normal saline at a concentration of 12.5 mg/ml such that injection of 0.2 ml corresponded to a dose of 100 mg/kg in a 25-g mouse. A single intraperitoneal injection of CA4DP at 100 mg/kg has been shown to be nontoxic and to elicit changes in tumor perfusion without significantly affecting tumor growth rates [11,25,29]. CA4DP solutions were stored in the dark at +4°C and used within 1 week of preparation.

Laser Doppler Flowmetry (LDF) Perfusion Measurement

LDF was carried out using a setup with a Periflux Laser Doppler flowmeter 4001 (Perimed, Stockholm, Sweden) and a custom-built probe with four integrated laser/receiver units

(outer diameter, 6 mm; fiber separation, 250 μm; time constant, 0.2 s; PF415-175; Perimed). Laser light at a wavelength of 780 nm was transmitted into the skin from the 42°C heated probe. The probe was held steady in the desired position by a micromanipulator. LDF signal was recorded continuously for 2 to 3 minutes while the calculated perfusion in arbitrary perfusion units (PU) was monitored graphically. During recording, care was taken to position the probe such that respiratory movements did not influence the readings (this was determined from the graphic representation of measured PU values). As soon as steady state had been reached (typically in 60–90 seconds), three individual values were noted from the display. The median of these three values was used for further data analysis. The LDF measurement apparatus was calibrated to 250 PU in a “motility standard” reference solution (Perimed) before the measurements, and calibration was regularly confirmed. Calibration was stable over time.

IFP Measurements

IFP was measured by the wick-in-needle technique [30], with minor modifications [31]. A 23-gauge sensing needle with a 2-mm side hole was inserted into the central part of the tumor. The needle remained in the same position during the entire experiment, ensuring that all time points would be equally affected in case of spatial heterogeneity within the tumor. The sensing needle was coupled to a pressure sensor by a water column in a tubing (inner diameter, 0.58 mm). This pressure sensor was connected to a MacLab/4e digitizer (ADInstruments Ltd., Castle Hill, Australia) through an ML112 bridge amplifier (ADInstruments Ltd.). Pressure data from the sensor were collected from the MacLab/4e digitizer using a PC with PowerLab Chart software v. 4.2 (ADInstruments Ltd.). The pressure-sensing system was calibrated against a water column of predefined height before each experiment, and calibration stability was confirmed at the end of each experiment. Calibration remained stable over time.

General Experimental Design

All experiments were carried out in a temperature-stabilized room at 25°C. Animals were anesthetized by a subcutaneous injection of 0.3 ml of xylazine (Rompun; Bayer Healthcare, Kgs. Lyngby, Denmark) and ketamine (Ketalar; Pfizer, Sollentuna, Sweden) diluted 1:4:15 in isotonic normal saline.

In initial experiments of 90 minutes' duration, the drug (saline for controls) was administered exactly 10 minutes before anesthesia for the best possible vascular volume equilibration after drug injection (before the anesthetic effect sets in) and the longest possible duration of anesthesia. Measurements were started 15 minutes after treatment. LDF was measured at 15-minute intervals, and the IFP of the same tumors was recorded continuously until the end of the experiment.

To investigate acute effects within the first 15 minutes of treatment, we conducted a second series of experiments. In these experiments, anesthesia was administered exactly 30 minutes before drug administration, and IFP was recorded

⁴A single dose of 100 mg/kg CA4DP was found to significantly decrease IFP in tumors sized ~ 300 mm³ and in ~ 800 mm³ of the highly sensitive KHT sarcoma. However, IFP was decreased only in ~ 300-mm³ tumors of the C3H tumor line, and not in ~ 800-mm³ tumors. This seemed attributable to lower IFP levels in large untreated C3H tumors, compared to the other untreated groups.

continuously, from 10 minutes before treatment to 30 minutes after treatment. Animals were sacrificed at the end of the experiments.

Statistical Analysis

Statistical significance was determined by two-tailed Mann-Whitney *U* test, which was performed using SPSS for Windows (SPSS Science, Chicago, IL). $P < .05$ was accepted as statistically significant.

Results

CA4DP Decreases Tumor Perfusion Measured By LDF

Perfusion was measured at 15-minute intervals, 15 to 90 minutes after CA4DP treatment. As shown in Figure 1, tumor perfusion was reduced at all posttreatment time points, following CA4DP treatment. In control mice, tumor perfusion remained stable at around 105 arbitrary PU throughout the experiment. The mean perfusion of treated tumors had dropped to 59 PU at 30 minutes after treatment (treated, $n = 13$; control, $n = 9$; $P = .004$), continued to decrease until 60 minutes after treatment, and then remained stable at around 37 PU. Prolonged measurements of some of the CA4DP-treated tumors showed that this stabilization in tumor perfusion persisted for at least 3 hours after treatment (data not shown).

CA4DP Decreases Tumor IFP

Tumor IFP was measured at 15-minute intervals, 15 to 90 minutes after CA4DP treatment. Treated tumors had IFP lower than that of control tumors at all time points (Figure 2). By 45 minutes after treatment, IFP had been significantly reduced to 72% of control levels (treated, $n = 13$; control, $n = 9$; $P = .043$). No further IFP reductions were observed at later time points. Three hours after treatment, IFP levels of approximately 70% of controls have been observed (Nielsen

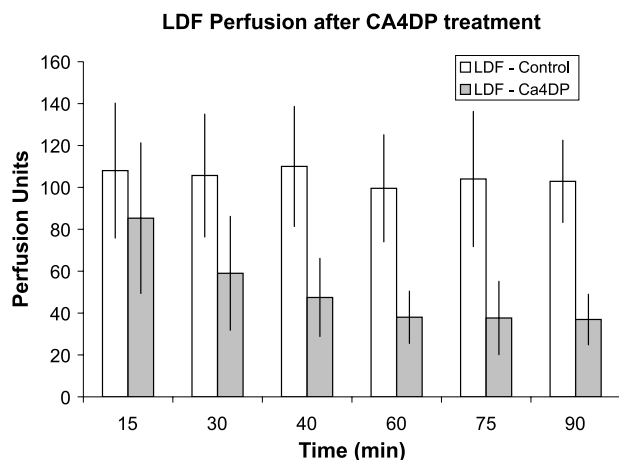


Figure 1. LDF perfusion measurements after treatment with 100 mg/kg CA4DP (mean \pm SD). CA4DP significantly reduced tumor perfusion 30 minutes after treatment (treated, $n = 13$; control, $n = 9$; $P = .004$). Tumor perfusion stabilized at around 37 PU at about 60 minutes after treatment.

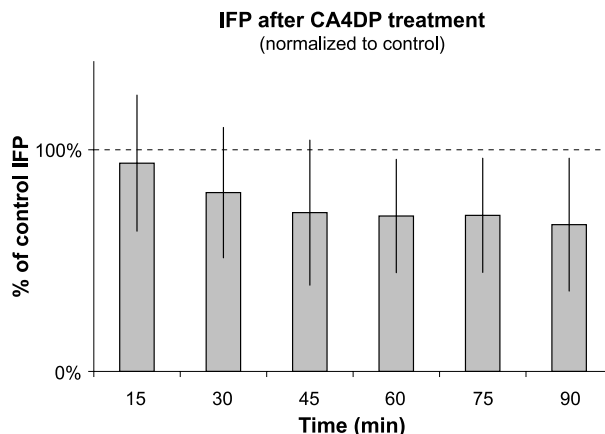


Figure 2. Tumor IFP after treatment with 100 mg/kg CA4DP. For clarity, measurements were normalized to the control level at the relevant time point; error bars represent SD. IFP in the treated group is significantly decreased from 45 minutes after treatment onward (treated, $n = 13$; control, $n = 9$; $P < .05$).

et al., unpublished observations), which indicates that IFP does not recover within that period.

IFP measurements were normalized to control levels at the relevant time point to highlight changes in IFP caused by the CA4DP treatment. Temporal fluctuations in IFP and tumor blood flow in treated and untreated tumors have been observed by other investigators [15]. Anesthesia-induced changes in heart rate and peripheral vasoconstriction can cause modest elevations in IFP, as seen in both treated and untreated animals at later experimental time points (absolute IFP values from the experiment are shown in Table 1).

To evaluate acute (< 15 minutes posttreatment) changes elicited by CA4DP, IFP was measured immediately after drug administration in a second series of experiments (Figure 3). IFP in treated tumors was significantly lower than that in controls as early as 30 minutes after treatment (treated, $n = 12$; control, $n = 9$; $P = .015$). No increases in IFP were observed. To illustrate even subtle effects of the drug as well as possible, results are shown as percent pretreatment IFP (the shorter duration of these experiments permitted us to obtain measurements of baseline IFP levels before treatment). Absolute IFP values from this experiment are shown in Table 2.

Discussion

CA4DP has been shown to increase endothelial permeability in an *in vitro* model of vasculature [18] and in tumor

Table 1. Absolute IFP Values (Mean \pm SD) Measured 15 to 90 Minutes after CA4DP Treatment.

Time after CA4DP (minutes)	IFP (mmHg)	
	Control	CA4DP
15	13.1 \pm 2.8	12.3 \pm 4.0
30	12.3 \pm 2.9	10.0 \pm 3.6
45	12.3 \pm 3.3	8.9 \pm 4.0
60	13.2 \pm 5.3	9.2 \pm 3.4
75	13.3 \pm 3.2	9.3 \pm 3.4
90	15.3 \pm 5.1	9.2 \pm 3.9

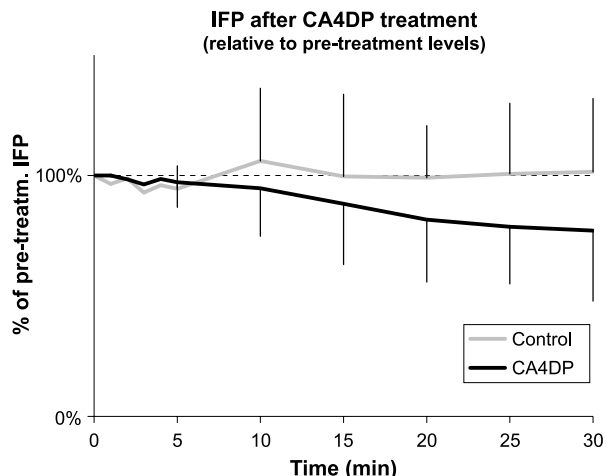


Figure 3. Continuous IFP measurements after CA4DP treatment (100 mg/kg), shown as percent pretreatment level (error bars represent SD; they were omitted at time points 1, 2, 3, and 4 minutes for clarity). Treated IFP is not significantly increased at any time point but is significantly decreased from 30 minutes after treatment onward (treated, $n = 12$; control, $n = 9$; $P < .05$).

vasculature *in vivo* [15]. Increasing vascular permeability may raise the hematocrit by augmenting the filtration of fluid and macromolecules, primarily albumin. This would increase blood viscosity, possibly decreasing blood flow through the tumor [15,18]. In addition, augmented extravasation could raise the extravascular content of macromolecules, further increasing hydrostatic pressure in tumor interstitial spaces, which in turn may increase IFP. Several authors propose that increased endothelial permeability might be the primary event in the action of CA4DP, increasing blood viscosity and transiently elevating IFP to an extent that could collapse—and thus occlude—tumor blood vessels [12,15,18,20].

Alternatively, it has been suggested that EC shape changes are, in fact, the primary event in reducing tumor perfusion, as the rounding up and protrusion of ECs into the vascular lumen may lead to obstruction. Rounding might also promote intratumoral thrombus formation due to contact between blood and the basal lamina, as well as EC detachment. EC shape changes occur with a similar time course as the *in vivo* effects of CA4DP on tumor blood flow, implying that they may be a key component of the mechanism [5,8,9,13,14]. Intravascular clotting, vessel obstruction, or both would cause a rapid decrease in perfusion. Interruption of blood supply would decrease microvascular pressure (MVP) inside large parts of the tumor vasculature, removing the source of fluid for filtration across the vascular membrane. As MVP is the principal driving force of IFP [31], IFP would drop in a rate defined by the diffusion of fluid out of the tumor periphery and other functional tumor drainage.

We found tumor perfusion to be significantly decreased by as early as 30 minutes posttreatment, thus confirming that CA4DP works as a vascular-disruptive agent in the C3H mammary carcinoma. This is in good agreement with previous studies of tumor perfusion (Kragh et al. [24]; Eikesdahl et al. [32]; Kristjansen et al., [21]) and measurements of red blood cell velocity [15] after CA4DP treatment. In our initial

IFP measurements, no increases in IFP were observed at 15 minutes posttreatment or later. On the contrary, IFP was significantly reduced compared to control levels at 45 minutes posttreatment and beyond. This indicates that the effect of CA4DP on tumor perfusion precedes significant modulation of IFP. These findings do not support the concept that vascular collapse is caused by IFP increases; however, a transient short-term elevation of IFP in the first 15 minutes after treatment cannot be excluded. We addressed this possibility with a short-term IFP experiment, in which IFP was monitored before treatment and immediately after. Again, no increases in IFP were detected. Therefore, decreased perfusion after CA4DP treatment must be caused by a different primary event, such as the EC shape changes described above. The effects of CA4DP on permeability, as demonstrated by other researchers [15], might not influence tumor IFP significantly because: 1) blood and interstitial space may be largely equilibrated beforehand as the tumor endothelium is already leaky; or 2) the increase in permeability is preceded or accompanied by a decrease in MVP.

Our failure to detect elevations in IFP after CA4DP treatment is consistent with the findings of Eikesdahl et al. [22], who did not detect changes in IFP. Perfusion was not assessed in that particular study, but the authors have previously reported decreased perfusion after treatment with CA4DP in the same experimental model [33]. Thus, CA4DP did have an effect on perfusion in that setting, and this effect was independent of any increase in IFP. We propose that the large size of the tumors in the study (3.8–19 cm³) may be the reason why IFP did not decrease after CA4DP treatment as it did in other experimental models; similarly, IFP decreases were not detected in very large C3H tumors treated with CA4DP (Nielsen et al., unpublished observations).

The effects of CA4DP and the more potent derivative AC7700 appear to differ in some aspects, but we consider the finding that AC7700 immediately and markedly decreased IFP [34] to be further evidence against the concept that combretastatins and their derivatives cause vascular collapse by modulating vascular permeability, thus increasing IFP.

In conclusion, we propose that reduced tumor perfusion after CA4DP treatment of the C3H mammary carcinoma

Table 2. Absolute IFP Values (Mean \pm SD) Measured before Treatment and Until 30 Minutes after CA4DP Treatment.

Time after CA4DP (minutes)	IFP (mmHg)	
	Control	CA4DP
Pretreatment	16.3 \pm 4.9	14.0 \pm 4.8
1	15.5 \pm 3.4	14.0 \pm 4.1
2	15.6 \pm 4.1	14.0 \pm 4.7
3	15.1 \pm 4.4	13.8 \pm 5.0
4	15.8 \pm 5.7	14.4 \pm 6.1
5	15.5 \pm 5.1	13.9 \pm 5.5
10	16.7 \pm 4.3	13.3 \pm 5.4
15	15.5 \pm 4.1	12.1 \pm 4.8
20	15.6 \pm 3.5	11.3 \pm 4.7
25	15.7 \pm 3.6	10.9 \pm 4.7
30	15.7 \pm 3.3	10.7 \pm 4.8

cannot be a consequence of vascular collapse caused by high IFP, as treatment decreased perfusion even in the absence of significant elevations in IFP. Our observation of significant reductions in IFP indicates that CA4DP decreases tumor perfusion by a mechanism that does not depend on increases in IFP.

Acknowledgement

CA4DP was generously supplied by Oxigene.

References

- [1] Pettit GR, Singh SB, Niven ML, Hamel E, and Schmidt JM (1987). Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from *Combretum cafrum*. *J Nat Prod* **50**, 119–131.
- [2] Chaplin DJ and Dougherty GJ (1999). Tumour vasculature as a target for cancer therapy. *Br J Cancer* **80** (1), 57–64.
- [3] Lin CM, Singh SB, Chu PS, Dempcy RO, Schmidt JM, Pettit GR, and Hamel E (1988). Interactions of tubulin with potent natural and synthetic analogs of the antimetabolic agent combretastatin: a structure–activity study. *Mol Pharmacol* **34**, 200–208.
- [4] Pettit GR, Temple C, Narayanan VL, Varma R, Simpson MJ, Boyd MR, Renner GA, and Bansal N (1995). Antineoplastic agents 322. Synthesis of combretastatin A-4 prodrugs. *Anticancer Drug Des* **10**, 299–309.
- [5] Chaplin DJ (2003). Drug based approaches for targeting tumor vasculature: development of combretastatin A4 phosphate. *Pathophysiol Haemost Thromb* **33** (1), 9–10.
- [6] el Zayat AA, Degen D, Drabek S, Clark GM, Pettit GR, and Von Hoff DD (1993). *In vitro* evaluation of the antineoplastic activity of combretastatin A-4, a natural product from *Combretum cafrum* (arid shrub). *Anticancer Drugs* **4**, 19–25.
- [7] Nabha SM, Wall NR, Mohammad RM, Pettit GR, and Al Katib AM (2000). Effects of combretastatin A-4 prodrug against a panel of malignant human B-lymphoid cell lines. *Anticancer Drugs* **11**, 385–392.
- [8] Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, and Chaplin DJ (1997). Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. *Cancer Res* **57**, 1829–1834.
- [9] Galbraith SM, Chaplin DJ, Lee F, Stratford MR, Locke RJ, Vojnovic B, and Tozer GM (2001). Effects of combretastatin A4 phosphate on endothelial cell morphology *in vitro* and relationship to tumour vascular targeting activity *in vivo*. *Anticancer Res* **21**, 93–102.
- [10] Iyer S, Chaplin DJ, Rosenthal DS, Boulares AH, Li LY, and Smulson ME (1998). Induction of apoptosis in proliferating human endothelial cells by the tumor-specific antiangiogenesis agent combretastatin A-4. *Cancer Res* **58**, 4510–4514.
- [11] Chaplin DJ, Pettit GR, and Hill SA (1999). Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. *Anticancer Res* **19**, 189–195.
- [12] Tozer GM, Kanthou C, and Baguley BC (2005). Disrupting tumour blood vessels. *Nat Rev Cancer* **5**, 423–435.
- [13] Kanthou C and Tozer GM (2002). The tumor vascular targeting agent combretastatin A-4-phosphate induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells. *Blood* **99**, 2060–2069.
- [14] Grosios K, Holwell SE, McGown AT, Pettit GR, and Bibby MC (1999). *In vivo* and *in vitro* evaluation of combretastatin A-4 and its sodium phosphate prodrug. *Br J Cancer* **81**, 1318–1327.
- [15] Tozer GM, Prise VE, Wilson J, Cemazar M, Shan S, Dewhurst MW, Barber PR, Vojnovic B, and Chaplin DJ (2001). Mechanisms associated with tumor vascular shut-down induced by combretastatin A-4 phosphate: intravital microscopy and measurement of vascular permeability. *Cancer Res* **61**, 6413–6422.
- [16] Parkins CS, Holder AL, Hill SA, Chaplin DJ, and Tozer GM (2000). Determinants of anti-vascular action by combretastatin A-4 phosphate: role of nitric oxide. *Br J Cancer* **83**, 811–816.
- [17] Brooks AC, Kanthou C, Cook IH, Tozer GM, Barber PR, Vojnovic B, Nash GB, and Parkins CS (2003). The vascular targeting agent combretastatin A-4-phosphate induces neutrophil recruitment to endothelial cells *in vitro*. *Anticancer Res* **23**, 3199–3206.
- [18] Tozer GM, Prise VE, Wilson J, Locke RJ, Vojnovic B, Stratford MR, Dennis MF, and Chaplin DJ (1999). Combretastatin A-4 phosphate as a tumor vascular-targeting agent: early effects in tumors and normal tissues. *Cancer Res* **59**, 1626–1634.
- [19] Beaugard DA, Hill SA, Chaplin DJ, and Brindle KM (2001). The susceptibility of tumors to the antivascular drug combretastatin A4 phosphate correlates with vascular permeability. *Cancer Res* **61**, 6811–6815.
- [20] Tozer GM, Kanthou C, Parkins CS, and Hill SA (2002). The biology of the combretastatins as tumour vascular targeting agents. *Int J Exp Pathol* **83**, 21–38.
- [21] Kristjansen PE, Kragh M, Murata R, Quistorff B, and Horsman MR (2001). Early changes in vascular physiology of solid tumors following combretastatin A-4 disodium phosphate treatment. *Abstract presented at 2001 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics*.
- [22] Eikesdal HP, Landuyt W, and Dahl O (2002). The influence of combretastatin A-4 and vinblastine on interstitial fluid pressure in BT4An rat gliomas. *Cancer Lett* **178**, 209–217.
- [23] Zhao D, Jiang L, Hahn EW, and Mason RP (2005). Tumor physiologic response to combretastatin A4 phosphate assessed by MRI. *Int J Radiat Oncol Biol Phys* **62**, 872–880.
- [24] Kragh M, Quistorff B, Horsman MR, and Kristjansen PE (2002). Acute effects of vascular modifying agents in solid tumors assessed by non-invasive laser Doppler flowmetry and near infrared spectroscopy. *Neoplasia* **4**, 263–267.
- [25] Murata R, Siemann DW, Overgaard J, and Horsman MR (2001). Interaction between combretastatin A-4 disodium phosphate and radiation in murine tumors. *Radiother Oncol* **60**, 155–161.
- [26] Murata R, Overgaard J, and Horsman MR (2001). Comparative effects of combretastatin A-4 disodium phosphate and 5,6-dimethylxanthone-4-acetic acid on blood perfusion in a murine tumour and normal tissues. *Int J Radiat Biol* **77**, 195–204.
- [27] Kragh M, Quistorff B, and Kristjansen PE (2001). Quantitative estimates of angiogenic and anti-angiogenic activity by laser Doppler flowmetry (LDF) and near infra-red spectroscopy (NIRS). *Eur J Cancer* **37**, 924–929.
- [28] Overgaard J (1980). Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue *in vivo*. *Int J Radiat Oncol Biol Phys* **6**, 1507–1517.
- [29] Murata R, Overgaard J, and Horsman MR (2001). Combretastatin A-4 disodium phosphate: a vascular targeting agent that improves that improves the anti-tumor effects of hyperthermia, radiation, and mild thermoradiotherapy. *Int J Radiat Oncol Biol Phys* **51**, 1018–1024.
- [30] Fadnes HO, Reed RK, and Aukland K (1977). Interstitial fluid pressure in rats measured with a modified wick technique. *Microvasc Res* **14**, 27–36.
- [31] Boucher Y and Jain RK (1992). Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse. *Cancer Res* **52**, 5110–5114.
- [32] Eikesdal HP, Schem BC, Mella O, and Dahl O (2000). The new tubulin-inhibitor combretastatin A-4 enhances thermal damage in the BT4An rat glioma. *Int J Radiat Oncol Biol Phys* **46**, 645–652.
- [33] Eikesdal HP, Bjerkvig R, Mella O, and Dahl O (2001). Combretastatin A-4 and hyperthermia; a potent combination for the treatment of solid tumors. *Radiother Oncol* **60**, 147–154.
- [34] Hori K and Saito S (2003). Microvascular mechanisms by which the combretastatin A-4 derivative AC7700 (AVE8062) induces tumour blood flow stasis. *Br J Cancer* **89**, 1334–1344.