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Tumour vascular disrupting agents: combating treatment resistance

[1]G M TOZER, PhD, [1]C KANTHOU, PhD, [2]G LEWIS, [2]V E PRISE, BSc, [2]B VOJNOVIC, PhD and [2]S A HILL, PhD

[1]*University of Sheffield, Academic Unit of Surgical Oncology, K Floor, School of Medicine & Biomedical Sciences, Beech Hill Road, Sheffield S10 2RX, UK and* [2]*Oxford University Gray Cancer Institute, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, UK*

ABSTRACT. A large group of tubulin-binding microtubule-depolymerizing agents act as tumour vascular disrupting agents (VDAs). Several members of this group are now in clinical trials in combination with conventional anticancer drugs and radiotherapy. Here we briefly update on the development of tubulin-binding combretastatins as VDAs, summarize what is known of their mechanisms of action and address issues relating to treatment resistance, using disodium combretastatin A-4 3–O-phosphate (CA-4-P) as an example. Characteristically, VDAs cause a rapid shutdown of blood flow to tumour tissue with much less effect in normal tissues. However, the tumour rim is relatively resistant to treatment. Hypoxia (or hypoxia reoxygenation) induces upregulation of genes associated with angiogenesis and drug resistance. It may be possible to take advantage of treatment-induced hypoxia by combining with drugs that are activated under hypoxic conditions. In summary, VDAs provide a novel approach to cancer treatment, which should effectively complement standard treatments, if treatment resistance is addressed by judicious combination treatment strategies.

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There are several groups of compounds being developed as tumour vascular disrupting agents (VDAs). Conceptually distinct from anti-angiogenic therapy, vascular disrupting therapy is aimed at causing a rapid and catastrophic shutdown of the established tumour vasculature, which thereby induces secondary tumour cell death. The VDA that is most advanced in development is DMXAA (5,6-dimethylxanthenone-4-acetic acid, also known as ASA404), which was developed from flavone acetic acid [1, 2]. Recently, DMXAA has been shown to extend survival of non-small cell lung cancer patients from 5.5 months to 14.0 months, when combined with conventional chemotherapy (www.antisoma.com). Tubulin-binding microtubule-depolymerizing agents are by far the largest group of VDAs and are structurally distinct from DMXAA. Disodium combretastatin A-4 3–O-phosphate (CA-4-P or Zybrestat^{TM}) is the lead compound of this group and is currently in Phase II/III clinical trials against a range of malignancies, in combination with conventional chemotherapeutic agents and radiotherapy. Table 1 summarizes the range of VDAs currently in clinical trial.

We have recently reviewed knowledge of the mechanisms of action and developmental status of CA-4-P and DMXAA [2]. Following a brief historical perspective and update on progress with the combretastatins, the main aim of the current paper is to investigate potential treatment resistance to VDAs and methods for overcoming it, focusing on CA-4-P as a model VDA.

Address correspondence to: Gillian M Tozer, University of Sheffield, Academic Unit of Surgical Oncology, K Floor, School of Medicine & Biomedical Sciences, Beech Hill Road, Sheffield S10 2RX, UK. E-mail: g.tozer@sheffield.ac.uk

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Historical perspectives

The modern concept of targeting the established tumour vasculature for indirect killing of tumour cells arose from the gradual recognition that established tumour blood vessels were both functionally and morphologically different from those in normal tissues. A key finding was that endothelial cells lining blood vessels in rodent tumours proliferate at a much higher rate than those in normal tissues [3]. This was later confirmed for human tumours [4]. Denekamp [5, 6] subsequently proposed a vascular targeting approach to cancer therapy based on this finding and investigated the extent of vascular shutdown necessary to induce substantial tumour cell death. At around the same time in the 1980s, approximately 10 years after Folkman's ground-breaking description of tumour angiogenesis [7], a vascular mode of action was ascribed to various emerging cancer treatments such as hyperthermia, photodynamic therapy, cytokines and flavone acetic acid. Vascular damage was strongly implicated because of extensive haemorrhagic necrosis (typical of the response to vessel ligation) and a poor tumour cell yield from excised animal tumours subjected to enzymatic digestion. These new cancer treatments contributed to the general interest in the tumour vasculature as a relatively unexplored target. Figure 1 illustrates some of the characteristics of the tumour microcirculation, which makes it abnormal and hence a source of potential molecular targets for therapy. Identification of specific molecular targets on tumour vasculature that could be used as a basis for therapy soon became a major research interest. Burrows and Thorpe [8] proved the principle of tumour vascular disruption in mice by subcutaneously implanting neuroblastoma cells expres-

Table 1. Vascular disrupting agents in clinical trial

Drug	Web-site	Drug type
Zybrestat™	www.oxigene.com/	CA-4-P, tubulin-binding agent
OXI 4503	As above	CA-1-P, tubulin-binding agent
AVE8062	www.aventisoncology.com/	Synthetic combretastatin
ABT751	www.abbott.com/	Sulphonamide β-tubulin inhibitor
TZT-1027	www.daiichi.co.uk/	Tubulin-binding agent
Trisenox™	www.trisenox.com/	Arsenic trioxide
NPI-2358	www.nereuspharm.com/	From marine fungus, tubulin binding
ASA404	www.antisoma.com/	DMXAA, flavonoid
Exherin™	www.adherex.com/	Peptide N-cadherin antagonist

DMXAA, 5,6-dimethylxanthenone-4-acetic acid, also known as ASA404, now licensed to Novartis AG.

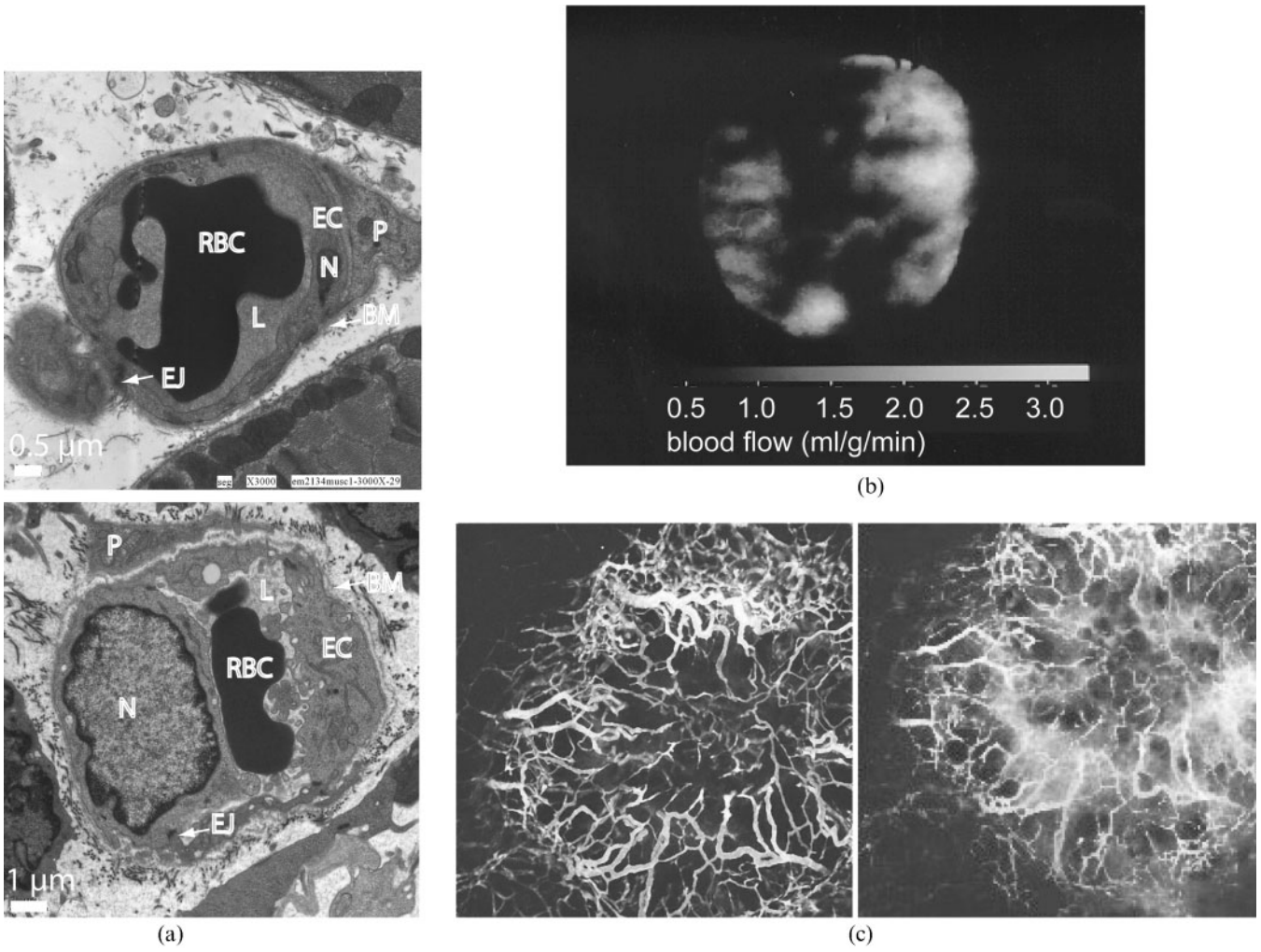

Figure 1. Characteristics of the tumour microcirculation. (a) Electron micrographs of a microvessel within the P22 rat sarcoma (bottom panel) and normal rat skeletal muscle (top panel). Note highly invaginated luminal surface of endothelial cells, large nucleus, very restricted lumen, disorganized basement membrane and poor contact between pericyte and endothelial cell, in the tumour compared with the normal vessel. (b) Blood flow rate in the P22 rat sarcoma, showing highly heterogeneous spatial distribution of flow across a tumour section (approximately 10 mm in diameter). Blood flow was estimated from the relationship between tissue uptake of intravenously administered ^{14}C-labelled iodo-antipyrine and time course of the tracer in arterial blood. Tissue levels of radioactivity were measured using autoradiography. (c) Complex vascular architecture of an HT29 human colorectal carcinoma growing as a xenograft in a dorsal skin-flap ''window'' chamber in an immunocompromised mouse, also showing rarefaction of the vascular bed towards the centre of the tumour (tumour approximately 3 mm in diameter). Vascular contrast was achieved by intravenous injection of fluorescently labelled 40 kDa dextran, monitored by multiphoton fluorescence microscopy. Left panel: a few minutes after administration of dextran. Right panel: approximately 15 min later, showing extensive leakage of dextran into the interstitial space, indicative of high vascular permeability. EC, endothelial cell; P, pericyte; BM, basement membrane; RBC, red blood cell; L, lumen; EJ, endothelial cell junction; N, nucleus of endothelial cell.

sing the inflammatory cytokine interferon-γ into mice. This induced major histocompatibility complex (MHC) class II expression on the tumour endothelium, which was then targeted by using antibodies to mouse MHC class II coupled to the toxin ricin. This led to the eradication of large solid tumours, thus demonstrating the potential for vascular disrupting approaches in cancer therapy. Recent progress in the search for specific molecular signatures on tumour vasculature, with potential for treatment targeting, is reviewed by Neri and Bicknell [9].

Development of the combretastatins

Sporadic reports of tubulin-binding agents having vascular effects have appeared over many years [10–14]. A systematic study of the tumour vascular effects following treatment with both newly discovered and established tubulin-binding agents was carried out by Chaplin et al [15] in the 1990s. The tubulin depolymerizing combretastatin, combretastatin A-4 (CA-4), emerged as a promising vascular disrupting agent from this study.

17 combretastatins had been isolated from the Cape Bushwillow tree *Combretum caffrum* by Professor Bob Pettit at Arizona State University. A soluble sodium phosphate salt of CA-4 (CA-4-P) was later developed [16], which is readily administered *in vivo* and rapidly cleaved to CA-4 by the action of endogenous non-specific phosphatases. Colchicine and CA-4 are structurally related and the two agents bind to tubulin at or close to the same site. The tumour vascular damaging effects of CA-4-P were first described in 1997 [17] and this compound is now being developed as Zybrestat^{TM} by OXiGENE Inc., made possible by its relative lack of toxicity compared with colchicine. This moderate toxicity is most likely owing to a short plasma half-life for CA-4 and reversible binding kinetics, as opposed to the pseudo-irreversible binding of colchicine. Tissues are therefore exposed to the drug for a relatively short time, which is nevertheless sufficient to cause vascular shutdown in susceptible tumour blood vessels. In animal models, this can be achieved within 20 min of drug exposure [18].

A second combretastatin, combretastatin A-1 (CA-1) [19], is also being developed as the sodium phosphate salt, CA-1-P, by OXiGENE Inc. (designated OXI4503). In pre-clinical models, CA-1-P is more potent than CA-4-P [20, 21] and both compounds are currently being tested in clinical trials. A synthetic derivative of the combretastatins, the Aventis Pharma compound AVE8062, is also in clinical trials. This is a pro-drug, the serine of which is cleaved by aminopeptidases, to form the active component [22]. Other synthetic analogues of the combretastatins are at much earlier stages of development.

Clinical trials of CA-4-P were initiated in 1998, followed shortly by trials of a related compound, ZD6126, and AVE8062. In Phase I/II clinical trials, imaging techniques (contrast-enhanced proton magnetic resonance imaging and positron emission tomography) were used to measure tumour and normal tissue uptake kinetics of contrast agents, as a measure of vascular response [23–30]. The results from three Phase I trials for CA-4-P have been reviewed [31]. These data confirmed the tumour selectivity of these agents in the clinical setting. However, in a majority of patients, the vascular parameters returned to baseline by 24 h after treatment, highlighting the need for optimization of dose schedules and/or more effective agents. The results for CA-4-P are consistent with animal studies performed at clinically relevant doses [27, 32]. Clinical trials of VDAs have now progressed to Phase II, in combination with conventional chemotherapy and radiotherapy. In addition, there is a Phase II/III trial of CA-4-P against anaplastic thyroid cancer, in combination with conventional chemotherapy, and a Phase Ib trial of CA-4-P in combination with the anti-angiogenic agent, bevacizumab (Avastin^{TM}) (www.oxigene.com).

Mechanisms of action

Within minutes of drug administration, VDAs cause a significant decrease in tumour blood flow with maximal effects between 1h and 6 h (Figure 2). Drug-induced vascular endothelial cell death is too slow a process to account for these rapid changes, but for CA-4-P *in vivo* effects are paralleled by very rapid remodelling of the actin cytoskeleton of endothelial cells *in vitro*, which is triggered by disruption of interphase microtubules following drug binding [33]. Endothelial cells are particularly sensitive to this compound and effects include rounding up of cells, assembly of actin stress fibres and actinomyosin contractility, formation of focal adhesions, disruption of cell–cell junctions, including those involving N- and VE-cadherin, and an increase in monolayer permeability to macromolecules [33, 34]. In a subpopulation of cells, additional effects involve F-actin accumulation into surface blebs, with cells rounding up and stress fibres misassembling into a spherical band surrounding the cytoplasm, accompanied by malformed focal adhesions. Signalling pathways associated with these processes involve the GTPase, Rho-A and Rho kinase and stress-activated protein kinase-2/p38 (SAPK/p38) [33], as reviewed by Kanthou and Tozer [35].

In vivo, CA-4-P increases tumour vascular permeability to macromolecules [36]. This is consistent with the *in vitro* effects and may lead to a decrease in blood flow because of an increase in viscous resistance to flow owing to fluid loss from blood to tissue. Previously, we have suggested that an increase in vascular permeability to macromolecules could also lead to blood flow shutdown via a transitory increase in tumour interstitial fluid pressure (IFP) [2]. However, direct measurements of tumour IFP, in the C3H mammary tumour model using the ''wick-in-needle'' method, have now shown that IFP does not increase at any time after CA-4-P treatment [37]. This does not rule out the possibility of an increase in the *differential* between IFP and intravascular capillary pressure following VDA treatment, leading to vascular collapse, which would occur if intravascular pressure decreases. This is suggested by upstream arteriolar vasoconstriction induced by CA-4-P and AVE8062 [18, 38]. Arteriolar vasoconstriction and cytoskeletal remodelling of endothelial cells following VDA treatment, as reported *in vitro*, may also contribute to an increase in

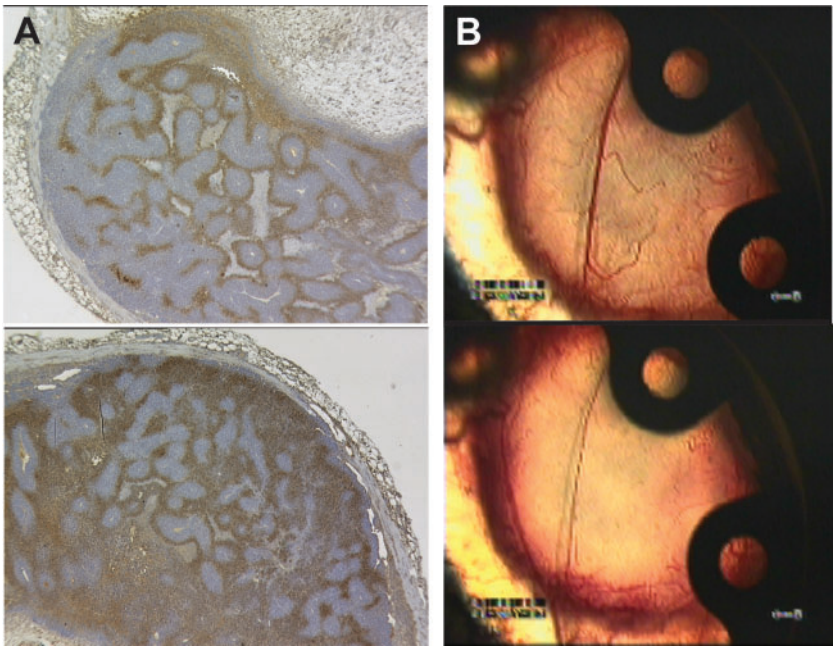

Figure 2. Effect of CA-4-P on tumour oxygenation and vascularity. (a) Mice bearing CaNT mammary carcinomas were treated with 50 mg kg^{-1} intraperitoneal (ip) CA-4-P and tumour hypoxia was identified by the intravenous injection of pimonidazole (OxyProbe^{TM}) and subsequent tissue processing. The top panel shows the control (untreated tumour) and the bottom panel the tumour at 1 h following CA-4-P administration. Increased pimonidazole staining is observed, indicative of substantial hypoxia induction. (b) Rats bearing P22 sarcomas in dorsal skin-flap ''window'' chambers were treated with 100 mg kg^{-1} ip CA-4-P and vascularity monitored by conventional transmitted light microscopy. The top panel shows the control (untreated tumours) and the bottom panel the tumour at 1 h 40 min following CA-4-P administration. Rapid loss of a large fraction of the visible vasculature and resistance of the tumour rim is observed.

geometric resistance to blood flow *in vivo*. As the blood flow falls below a critical level, red cells stack together to form rouleaux, increasing viscous resistance to flow and further blood stagnation [39]. Sustained vascular damage leads to haemorrhage into tumour tissue, coagulation and tumour infiltration by immune effector cells such as neutrophils and macrophages. In one study, CA-4-P enhanced the immune response of rats bearing intrahepatic colon carcinomas [40]. The generality of this finding is unknown but it will be important to determine the ultimate effect of infiltrating immune effector cells on treatment outcome with CA-4-P, as macrophages in the tumour microenvironment have important pro-angiogenic effects.

Extended exposure to CA-4-P can affect endothelial cell proliferation and migration and is clearly toxic to cells *in vitro*. Therefore, if drug exposures are sufficient, VDAs can be anti-angiogenic as well as vascular disrupting, and endothelial (and tumour) cell death could contribute to treatment outcome. Death can occur by several means. Firstly, extensive disruption of interphase microtubules in endothelial cells can lead to a relatively rapid form of necrotic cell death, in which the blebbing morphology described above is an early manifestation [33]. There is also evidence that disruption of VE-cadherin junctions by CA-4-P is associated with a cell death pathway mediated by inhibition of PI3K/Akt signalling [34]. In proliferating endothelial cells, death is associated with damage to mitotic spindles, which are at least as sensitive to CA-4-P as interphase microtubules [41]. Most investigators agree that the primary cell death pathway in proliferating endothelial cells occurs via a caspase-independent mechanism, which is nevertheless linked to apoptosis and dependent upon mitotic arrest. Death occurs as cells attempt to leave mitosis, such that drug exposures of many hours are required for significant cells to accumulate in mitosis and subsequently die.

Treatment resistance

Susceptibility to VDAs

Tumour blood vessels are generally extremely susceptible to CA-4-P [42] and this was the basis for combretastatins entering clinical trials. Many tumours are characterized by regions of necrosis and hypoxia, which suggest that the blood supply is barely adequate to support tumour growth. Indeed tumour blood flow is characteristically highly heterogeneous (Figure 1), with blood in some vessels being practically stationary and/or periodically reversing in flow direction. Morphologically too, tumour blood vessels appear fragile and susceptible to disruption. They are often sinusoidal in appearance, with poor development of the vascular wall, comprising of endothelial cells with poor cell–cell contacts and abnormal basement membrane. Mural support cells, in the form of pericytes, may be deficient and often make poor contact with endothelial cells (Figure 1).

However, at the pre-clinical level, there is variation in response to VDAs between different tumour types. Knowledge of the factors that predict blood vessel susceptibility to CA-4-P and other combretastatins will enable selection of appropriate patients for current VDA treatment and open up new avenues for research into novel pathways for targeting the established tumour vasculature. Susceptibility may occur at the cellular or tissue level and putative factors determining susceptibility are shown in Table 2.

There is evidence that vascular permeability to macromolecules correlates with the response to CA-4-P [48] and this may relate to the pre-treatment level of interstitial fluid pressure. We have recently shown that expression of a high-molecular-weight splice variant of VEGF-A (VEGF188) in a mouse tumour model is uniquely associated with pericyte recruitment and is also highly resistant to CA-4-P [51]. Thus endothelial

Table 2. Determining factors for tumour susceptibility to microtubule depolymerizing VDAs

Cellular level	References	Tissue level	References
High endothelial (and tumour) cell proliferation rate increase susceptibility to cell killing	[3, 4]	Regional instabilities in blood flow may make any further drug-induced decrease in flow catastrophic	[43, 44]
Differential expression of tubulin isotypes, tubulin mutations, post-translational modifications of tubulin and types of MAPs may influence microtubule disruption	[45]	High vascular permeability and interstitial fluid pressure may make blood vessels prone to collapse if there is a further acute increase in vascular permeability	[46–48]
Defective cell–cell junctions may sensitize to further junctional disruption	[33, 34]	Immaturity of the vascular wall, *e.g.* poor contact between endothelial cells and pericytes, may reduce stability of tumour blood vessels following endothelial cell damage	[49]
Hypoxia and hypoglycaemia influence signalling events	[50]	Self-trapping of VDA as tumour blood flow decreases increased drug exposure	[42]

MAP, microtubule-associated protein; VDA, vascular disrupting agent.

cell–pericyte interactions are likely to be important for the resistance of normal tissues to the effects of VDAs.

A potential advantage of all vascular-targeted strategies is that cellular components of the tumour vasculature are less susceptible than tumour cells to mutations that give rise to drug resistance. However, there are various sources of treatment resistance to VDAs that need to be overcome and these are addressed below.

Toxicity

In clinical trials, dose-limiting toxicities for CA-4-P included dyspnoea, myocardial ischaemia, reversible neurological events and tumour pain. Most common adverse events after a single intravenous injection of CA-4-P were mild (Grade 1 or 2) nausea, vomiting, headache, fatigue and tumour pain. CA-4-P did not cause haematological toxicity associated with other classes of tubulin-binding anticancer drugs such as the vinca alkaloids and taxanes.

Cardiovascular side effects of the tubulin binding VDAs have been the main concern in clinical trials. For CA-4-P, hypertension preceded three cases of reversible myocardial effects, which contributed to the establishment of the current maximum tolerated dose. Hypertension is most likely caused by vasoconstriction of normal blood vessels. The concomitant use of vasodilators with CA-4-P in rats, can eliminate the hypertensive effect without altering the blood flow shutdown effects in the tumour [52], suggesting that this may alleviate some of the clinical problems.

Resistance of the tumour rim

Well-tolerated doses of VDAs in mice can kill over 90% of tumour cells. However, tumours regrow from surviving cells in the tumour rim, which has proved exceptionally difficult to eradicate. Typically, a viable rim of tumour tissue a few cells wide persists to repopulate the tumour following VDA treatment at close to the maximum tolerated dose level in animal models (Figure 2). Resistance of the tumour rim, initially in terms of the primary blood flow reduction achieved and then in terms of the consequent extent of tumour necrosis, appears to be the case not only for tumours in animal models but also for human tumours [27]. This characteristic accounts for most of the resistance to VDAs. The extent of tumour growth delay following VDA treatment in animal models is dependent on type of tumour, dose and scheduling, but overall it is clear that none of the current agents is curative as single agents. However, there is encouraging evidence that VDAs will enhance conventional treatments. Combined efficacy can be achieved if the two treatments have independent targets and/or provide spatial co-operation, independent toxicities or potentiate each other's actions.

Clearly, the primary target of VDAs is different from conventional treatments designed to target tumour cells directly, and so it is hoped that VDA treatment kills some tumour cells that survive conventional treatment alone. Spatial co-operation could be achieved by combining VDA treatment with a modality that targets the tumour periphery. Conventional chemotherapeutic agents and radiation are effective against highly proliferating and well-oxygenated cells, which are most evident in the periphery of tumours. In addition, blood flow tends to be more efficient at the periphery, allowing ready access to blood-borne anticancer agents. The rationale for combining vascular disrupting agents with conventional treatments is therefore apparent and pre-clinical studies have indicated a benefit of combining various combretastatins with radiotherapy and a range of chemotherapeutic agents, most notably the platinum drugs and taxanes [53, 54]. There is some evidence, at least in mice, that these improvements in tumour response can be achieved without any increased toxicity [55].

Scheduling is an important and complex issue in combination treatments. Pre-clinical studies have generally concluded that it is inadvisable to give radiotherapy or chemotherapy shortly after VDA administration, when the blood flow is reduced to the tumour regions that are destined to survive the treatment. These areas will be hypoxic and therefore radioresistant and poorly accessible by blood-borne chemotherapeutic agents. However, VDA-induced reduction in tumour blood flow can be exploited to ''trap'' chemotherapeutic drugs in tumour tissue, thus providing potentiation of the drug effect. In most studies, it is difficult to separate this effect from any spatial co-operation or direct interaction of a VDA with a

second agent. However, at least in one case (the combination of 5-fluorouracil (5-FU) with CA-4-P) an effective tumour growth retardation has been achieved for a combined treatment, in the absence of any corresponding increase in tumour levels (trapping) of the drug [56]. In radiotherapy, damage to the tumour vasculature is increasingly recognized as being influential in determining tumour cell survival, and radiation-damaged tumour blood vessels may be particularly susceptible to VDAs. There is some evidence that CA-4-P and ZD6126 have a particular impact on the radiation-resistant hypoxic cell population but the mechanism behind this effect requires further investigation [57]. Recently, the clinical combination of CA-4-P and radiotherapy in a palliative setting for non-small cell lung cancer has shown some benefit in terms of tumour vascular effects, as measured by contrast-enhanced dynamic CT [58].

In addition to combining VDAs with conventional agents that have some selectivity for the tumour periphery, combination with novel biological anticancer agents also has significant potential. For instance, antibodies with potent binding characteristics are found to localize in the tumour periphery, with very little penetration into the tumour centre, resulting in poor efficacy as single agents. This is most likely a consequence of both poor delivery (low blood flow) and poor convective extravasation (high IFP) at the centre of tumours. Spatial co-operation of high-molecular-weight biologicals with VDAs has been found for the combination of radioimmunotherapy and CA-4-P or DMXAA in pre-clinical studies [59, 60] and this is now being tested in clinical trials.

Recent innovations in drug delivery systems may also impact on the resistant tumour rim. Blood flow reductions in tumour regions that are destined to survive the treatment are a concern in terms of subsequent drug delivery, as well as acting as a stimulus for expression of growth-enhancing genes. The development of nanoparticles that consist of a core and a pegylated lipid envelope, for timed release of two different drugs, has been described as one approach to tackling this problem [61]. In this case, vascular shutdown is instigated by release of CA-4 from the outer envelope and this is followed, once the nanoparticles are preferentially trapped in the tumour tissue, by release of a chemotherapeutic drug, doxorubicin, from the core. Another approach exploits CA-4 encapsulated in liposomes that incorporate specific peptide sequences on their surface for preferential targeting of irradiated tumour blood vessels via the integrin, $\alpha V\beta 3$ [62]. In this way, it is hoped to increase the selectivity of CA-4 to the tumour vasculature, when used in combination with radiotherapy.

The cause of the resistant tumour rim is not completely understood. There is some speculation that tumour cells residing in the tumour periphery acquire oxygen and nutrients from the surrounding undamaged normal tissue, thus surviving VDA treatment. However, this does not explain why blood flow is less compromised in these peripheral regions, in the first place (Figure 2). Two important factors are likely to be IFP and the vascular architecture in the two regions. Interstitial fluid pressure rises precipitously from the tumour periphery to the tumour centre [46], such that a decrease in intravascular capillary pressure at the tumour centre may be catastrophic, whereas it is tolerated at the periphery. Small-calibre vessels are also more sensitive to shutdown than larger ones and the proportion of these is often far higher at the centre than at the periphery. A complex vascular plexus often exists at the tumour periphery, compared with a rarefaction of the vascular bed at the tumour centre (Figure 2), so that in the event of extensive vascular damage a residual flow is likely to persist at the periphery rather than at the centre. Indeed, this situation is often directly observed using microscopic techniques.

Promotion of angiogenesis

The extensive ischaemic insult to tumours following VDA treatment results in severe tumour cell hypoxia even in the surviving tumour rim (see Figure 2) [63]. This raises the possibility of hypoxia- or hypoxia reoxygenation-induced angiogenesis, with the concern that regrowing tumours will be particularly aggressive. An increase in expression of both VEGF and basic fibroblast growth factor (bFGF) proteins in xenografted tumours, following CA-4-P and CA-1-P treatment, has been reported [64, 65]. In addition, both of these agents have been shown recently to increase the number of circulating endothelial progenitor cells in mice, which contribute to revascularization of tumours following VDA treatment [66]. These considerations may explain the good responses observed in pre-clinical models for the combination of VDAs with anti-angiogenic agents, such as the VEGF receptor tyrosine kinase inhibitor, ZD6474 [67] and nitric oxide synthase inhibitors [42]. In addition, infiltration of VDA treated tumour tissue by macrophages, presumably arising from chemoattractants derived from hypoxia or necrosis may contribute to tumour revascularization.

It may be possible to exploit the VDA-induced tumour ischaemia by combining VDA treatment with the so-called bioreductive drugs [68], or with other hypoxia-targeting strategies [69]. Bioreductive drugs are prodrugs that are activated to cytotoxic agents under hypoxic conditions. Oxygen protects the prodrug against reductive catabolism by cellular enzymes. In the absence of oxygen, the drug is reduced to an active form or the reduction process triggers release of an active effector molecule. An example is the combination of DMXAA with tirapazamine [70]. Figure 3 illustrates the potential of combining CA-4-P with either tirapazamine or another bioreductive drug, AQ4N (banoxantrone), which is metabolized to the stable DNA binding agent and topoisomerase II inhibitor, AQ4 (www.novacea.com) [71].

Development of drug-resistant tumour cells

A recent study in an animal model has also shown that CA-4-P can increase the surviving tumour cell expression of the glucose-regulated protein GRP78 [72]. GRP78 is an endoplasmic reticulum-associated chaperone molecule, which is inducible by severe glucose depletion, anoxia and acidosis, and is associated with drug resistance. This important finding illustrates the fact that the response of tumour cells to vascular disrupting agents can have a major influence on treatment outcome and points to the potential impact of VDA treatment on development of drug resistance, which warrants further investigation.

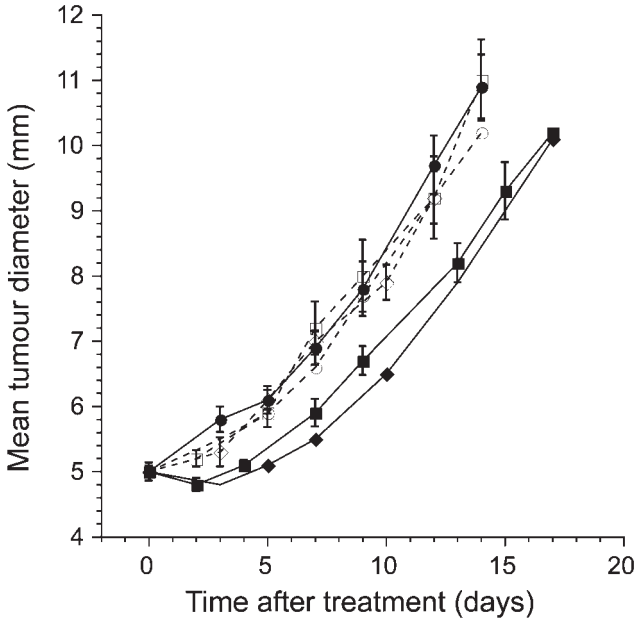

Figure 3. Advantage of combining the bioreductive drugs tirapazamine or AQ4N with CA-4-P on growth of the mouse mammary tumour, CaNT. Open symbols represent treatment with single agents (circles, CA-4-P 100 mg kg^{-1} ip; squares, tirapazamine 25 mg kg^{-1}ip; diamonds, AQ4N 50 mg kg^{-1} ip). Solid symbols represent combined treatment (circles, growth of control (untreated tumours); squares, tirapazamine plus CA-4-P 1 h later; diamonds, AQ4N plus CA-4-P simultaneously). None of the agents produced any significant growth delay when administered as single agents, whereas combination of either bioreductive drug with CA-4-P caused significant growth delay. Timings of drugs represent the optimum of several tested.

Summary

Vascular disrupting agents or VDAs, unlike anti-angiogenic agents, cause a rapid and catastrophic vascular collapse in tumour tissue, leading to extensive tumour cell necrosis. In addition, anti-angiogenic effects may be revealed in chronic dosing schedules. The actin cytoskeleton and integrity of cell–cell junctions are intimately involved in evoking vascular collapse. Extended vascular shutdown, which is necessary for tumour cell kill, requires a complex series of events involving coagulation and immune effector cells. Cardiovascular effects represent the most concerning toxicity. The tumour rim is resistant to VDA treatment and resistance may also occur via upregulation of genes that are associated with angiogenesis and tumour cell drug resistance. Several novel approaches have been investigated to address these problems. Despite these issues, VDAs have enhanced conventional therapy in the pre-clinical setting and hold the promise for effective complementation of conventional cancer treatments.

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