Combretastatin A4 phosphate

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Combretastatin A4 phosphate (CA4P) is a water-soluble prodrug of combretastatin A4 (CA4). The vascular targeting agent CA4 is a microtubule depolymerizing agent. The mechanism of action of the drug is thought to involve the binding of CA4 to tubulin leading to cytoskeletal and then morphological changes in endothelial cells. These changes increase vascular permeability and disrupt tumor blood flow. In experimental tumors, anti-vascular effects are seen within minutes of drug administration and rapidly lead to extensive ischemic necrosis in areas that are often resistant to conventional anti-cancer treatments. Following single-dose administration a viable tumor rim typically remains from which tumor regrowth occurs. When given in combination with therapies targeted at the proliferating viable rim, enhanced tumor responses are seen and in some cases cures. Results from the first clinical trials have shown that CA4P monotherapy is safe and reduces tumor blood flow. There has been some promising demonstration of efficacy. CA4P in combination with cisplatin is also safe. Functional imaging studies have been used to aid the selection of doses for phase II trials. Both dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and positron emission tomography can measure the anti-vascular effects of CA4P in humans. This review describes the background to the development of CA4P, its proposed mechanism of action, the results from the first clinical trials with CA4P and the role of imaging techniques in its clinical development. *Anti-Cancer Drugs* 15:179–187 © 2004 Lippincott Williams & Wilkins.

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Introduction

The development of a vasculature is a fundamental requirement for the growth of a solid tumor and the accumulation of genetic changes involved in the stimulation of angiogenesis is an integral part of tumorigenesis [1]. The neovasculature that develops is rapidly proliferating and fragile compared with normal tissue endothelia [2,3]. Nevertheless, each vessel is capable of supporting the growth of many thousands of tumor cells. Rapidly proliferating tumor cells can outstrip the growth of new vessels, become hypoxic, and eventually die from lack of oxygen and nutrients [4]. These hypoxic cells are resistant to many cancer treatments, such as radiation and some chemotherapeutic drugs. The limited penetration of anti-cancer agents from blood vessels into tumor tissues can also limit the success of therapy. The ability to target tumor vessels represents an exciting new development in an expanding armory of anti-cancer treatments. A drug that targets tumor endothelium should not be limited by drug penetration into tumor tissues. Such a drug would also not be limited by the presence of hypoxic cells in a tumor. Destruction of the blood vessels that support the continuing growth of a tumor can lead to rapid cascade tumor cell death from a lack of nutrients and oxygen in areas of tumors considered resistant to many anti-cancer agents.

Combretastatin A4 phosphate (CA4P) is undergoing phase II evaluation in cancer patients. It is one of the lead compounds of a new class of anti-cancer agents that target the tumor vasculature rather than rapidly dividing tumor cells. The structure and synthesis of CA4P was described in 1989, and the results from the first phase I trials published in 2002 and 2003. Functional imaging studies were an integral part of the preclinical development of CA4P driven by the search for methods to provide the proof of principle for the mechanism of action of the drug and its activity *in vivo*.

The development of CA4P

In 1987, Pettit *et al.* described the isolation, structure and synthesis of combretastatin A1 [5]. The drug was isolated from the Zulu medicinal tree *Cambretum caffrum*, the structure established and a method for its synthesis described. A series of 17 natural products and 22 synthetic agents were subsequently evaluated for their anti-mitotic and cytotoxic activity, ability to inhibit tubulin polymerization, and ability to inhibit the binding of colchicine to tubulin [6]. Combretastatin A4 [*cis*-1-(3,4,5-trimethoxyphenyl)-2-(3-hydroxy-4-methoxyphenyl)ethene; CA4] was the most promising agent and was reported to inhibit cell growth by 50% at 7 nM and to inhibit tubulin polymerization by 50% at 2.5 μM [6]. The

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isolation, structure and synthesis of CA4 was described in 1989 [7]. The limited water solubility of CA4 led to the synthesis of water-soluble prodrugs [8]. The potassium and sodium phosphate derivatives of CA4 were found to be suitable, and the sodium phosphate derivative selected for further preclinical development. Combretastatin A4 disodium phosphate (CA4P) is the compound that has been evaluated recently in clinical trials. It is a prodrug and under physiological conditions the phosphate group is cleaved from CA4P by endogenous non-specific phosphatases. The cytotoxicity profile of the prodrug and active compound are similar, indicating no loss of activity for CA4P [9].

The concept of vascular targeting

The concept of vascular targeting was originally outlined by Denekamp [10,11]. It is an approach that is distinct from anti-angiogenic strategies, which are aimed at preventing the growth of new blood vessels in a tumor. Anti-angiogenic strategies are expected to show the greatest efficacy with a protracted drug administration. In contrast, vascular targeting agents destroy the existing neovasculature of a tumor and aim to cause the selective shutdown of tumor blood vessels and extensive ischemic necrosis [12]. Vascular targeting agents are expected to show the greatest efficacy when used in combination with conventional anti-proliferative therapies. Selectivity of both strategies is conferred by the differences between tumor and normal tissue endothelium. Tumor vessels are more proliferative and fragile, and less mature than their normal tissue counterparts [2,3]. The overexpression of antigens, such as vascular endothelial growth factor, is also common [13].

At the end of the 1980s and start of the 1990s a number of agents were shown to have anti-vascular effects on tumors: flavone acetic acid [14–16], tumor necrosis factor-α [17], the tubulin-binding drugs colchicine, vincristine and vinblastine [18], and a tumor blood vessel specific antibody conjugated to a toxin [19]. Work by Baguley *et al.* led to the development of the flavone acetic acid analog, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), which is under clinical development [20–22]. Chaplin *et al.* investigated the anti-vascular effects of Vinca alkaloids [23] and other tubulin-binding agents including some combretastatins [24]. CA4P was subsequently shown to have potent and selective toxicity towards tumor vasculature [9].

The mechanism of action of CA4P

CA4 has a high affinity for tubulin [7,25] and destabilizes the tubulin polymers of the cytoskeleton. Thus, it is sometimes referred to as a tubulin depolymerizing or destabilizing agent. The work of Dark *et al.* [9] showed that CA4P caused rapid, extensive and irreversible vascular shutdown in experimental tumor models following the administration of a single dose at 1/10th the maximum tolerated dose (MTD). A reduction in vascular volume of 93% was measured 6 h following drug administration. In another study a significant increase in the hypoxic fraction of a mouse mammary carcinoma was measured 1 h after the injection of CA4P in tumor-bearing animals [26]. Rapid reductions in tumor blood flow can be measured. In one experimental tumor model a 100-fold reduction in flow was measured 6 h following drug administration to tumor-bearing animals [27]. Tumor vascular permeability has been shown to increase by approximately 160% just 1 min following drug administration [28].

CA4P was shown to be cytotoxic towards proliferating but not quiescent endothelial cells [9]. Although the cytotoxic effects of CA4P towards endothelial cells were reported to be mediated by the induction of apoptosis seen several hours following drug treatment [29], later studies suggested that only a small proportion of cells undergo apoptosis and that endothelial cells predominantly undergo mitotic catastrophe [30,31]. Nevertheless, the cytotoxic effects of CA4P seen several hours after drug exposure in both endothelial [9] and tumor [32] cells may not be important in the mechanism of action of the drug *in vivo* as the *in vivo* effects of CA4P are measurable within minutes of drug administration.

Non-cytotoxic concentrations of CA4P result in the disruption of actin filaments and tubulin microtubules of human endothelial cell cultures approximately 1 h after drug treatment [33]. Rapid changes in the cell shape of proliferating human endothelial cells also occur within minutes of CA4P exposure with recovery by 24 h [34]. Kanthou and Tozer [35] carried out a detailed study of the effects of CA4P on endothelial cell morphology and function. They showed that changes in the cytoskeleton of endothelial cells occurred within minutes of CA4P exposure, which were accompanied by changes in morphology as the cells retracted and intercellular gaps formed. Membrane blebbing was seen in some cells. The molecular processes underlying these changes in morphology were then investigated. Endothelial cell contraction is thought to result from the activation of actin–non-muscle myosin interactions regulated by myosin light chain in signaling pathways stimulated by growth factors, cytokines and stress-inducing agents. One of these pathways involves the phosphorylation of myosin light chain by Rho-kinase following its activation (as a result of growth factor or cytokine stimulation) by the guanosine triphosphatase Rho. The authors suggested that CA4P activates Rho/Rho-kinase leading to myosin light chain phosphorylation and increased actin polymerization/filament stabilization. These effects were associated with an increase in endothelial cell permeability, suggesting their involvement in the rapid response of tumor endothelia to CA4P.

CA4P is tumor selective rather than specific

The mechanisms underlying the selectivity of CA4P for tumors were outlined above (proliferating and fragile tumor versus normal tissue endothelial cells). In an animal model CA4P was shown to reduce tumor blood flow 6 h following administration by approximately 100-fold compared with approximately 7-fold in the spleen, the most sensitive of a series of normal tissues studied [27]. The lack of specificity for tumor endothelium is highlighted by studies showing a CA4-induced inhibition of the neovascularization of hyperplastic thyroids [36] and retina [37]. These studies suggest that CA4P may have future value in the treatment of some non-cancer diseases.

In vitro anti-tumor activity of CA4P

Early studies showed that CA4 inhibited the growth of murine lymphocytic leukemia and human colon cancer cell lines [7]. CA4 was also shown to be effective against two P388 cell lines with acquired resistance to daunorubicin [38]. CA4P showed activity towards a panel of malignant human B lymphoid cell lines [39], and time- and drug-dose dependent effects of CA4P were seen in human non-small cell lung cancer cell lines [32]. Although CA4P is clearly cytotoxic towards tumor cell lines, it is not known how much this direct toxicity contributes to its mechanism of action *in vivo*.

In vivo anti-tumor activity of CA4P

In vivo activity has been demonstrated towards a range of murine tumors [26,27,40] including mouse models of colorectal [41] and lung [42] metastases. The drug is active towards human xenografted tumors including non-Hodgkin's lymphoma [43], non-small cell lung cancer [32] and Kaposi's sarcoma [44]. Response to CA4P has been shown to vary between tumor models [45,46]. Studies have suggested that the drug is more active towards large rather than small tumors [47]. High nitric oxide synthase activity has been implicated in tumor resistance to CA4P [46], as has poor vascular permeability [45].

Interactions with other agents

Although the administration of CA4P to tumor-bearing animals results in rapid ischemic necrosis, non-clinical studies have shown that rapid regrowth occurs from a surviving viable rim of tumor. Single-dose administration of the drug is therefore typically associated with very little growth delay in experimental tumors. Options for improving the therapeutic efficacy of the drug have centered on the investigation of CA4P in combination with conventional anti-proliferative therapies (e.g. radiation, cisplatin). The rationale behind these studies is CA4P targets the central hypoxic parts of tumors, often considered resistant to conventional therapies, and that the anti-proliferative approaches target the viable rim. These studies are summarized in Table 1 and show that CA4P can enhance the response of experimental tumors to radiation and a number of widely used chemotherapeutic agents. Beneficial interactions with hyperthermia and radioimmunotherapy have also been reported.

Clinical studies with CA4P

Results have been published from two phase I clinical studies. A pharmacokinetic and translational study was carried out in the US in 25 patients with advanced cancer [48]. The drug was given as a 10- or 60-min infusion every 3 weeks with dose escalation from 18, 36, 60 to 90 mg/m^2. A reversible dose-limiting pulmonary toxicity was seen in two patients treated at the highest dose level. Cumulative side-effects were minimal with the exception of mild, or rarely moderate, fatigue. The toxicities observed were short-term and resolved within 24 h. They included flush, hot flashes, pruritus, nausea, vomiting, headache, abdominal cramps and tumor pain. A number of episodes of grade 1 QT_c interval prolongation were seen on electrocardiogram tracings. There were none of the side-effects commonly associated with cytotoxic chemotherapeutic drugs (myelosuppression, stomatitis, alopecia). Pharmacokinetic studies revealed a plasma half-life for CA4P of 0.47 h and 4.2 h for the active drug CA4. An average of 67% of the drug was excreted as the glucuronidated metabolite in the urine within 24 h of drug administration. A complete response was observed in a patient with a refractory metastatic anaplastic thyroid cancer. A complete resolution of a palpable left neck node was seen after 2 cycles of therapy and after 8 cycles there was complete resolution of neck abnormalities on computed tomography. Subsequent surgery to remove micrometastatic disease revealed a pathological complete remission. Following 2 further cycles of therapy the patient was free of disease a minimum of 30 months following the end of treatment. Another two patients had prolonged progression-free survival. A patient with metastatic colon carcinoma received 24 cycles over 19 months and a patient with metastatic medullary thyroid cancer received 15 cycles and was progression-free for 12 months. A patient with metastatic renal cancer had stabilized disease for 6 months. Finally, a patient with non-small cell lung cancer had a 34% reduction in their disease after 2 cycles of therapy. Doses of 60 mg/m^2 or less were considered to be the upper boundary of the MTD.

The other phase I pharmacokinetic study was carried out in the UK [49]. Thirty-four patients received weekly doses of a 10-min infusion of 5, 10, 20, 40, 52, 68, 88 or 114 mg/m^2 CA4P for 3 weeks followed by 1 week of rest. Intra-patient dose escalation was allowed in those not experiencing grade 2 or higher drug-related toxicity. The dose-limiting toxicities were a fatal ischemia in previously irradiated bowel at 52 mg/m^2, vasovagal syncope and motor neuropathy at 88 mg/m^2, and reversible ataxia at

Table 1 Studies investigating CA4P in combination with other anti-cancer agents

CA4P plus	Effect of CA4P	Reference
Radiation	Enhanced growth delay in CaNT tumors and resulted in cures in 50% of animals	Chaplin *et al.*, 1999 [69]
Radiation	Increased KHT murine sarcoma cell kill when given 0.5–1.0 h after radiation compared with that for radiation alone	Li *et al.*, 1998 [70]
Radiation	Enhanced the anti-tumor effects of radiation in KHT murine sarcoma	Li *et al.*, 2002 [71]
Radiation	Enhanced C3 H mouse mammary tumor response when given at the same time or 30–60 min after radiation	Horsman *et al.*, 2000, 2002 [72,73]
Radiation	No effect on small rhabdomyosarcoma tumor model; in large tumors increased growth delay	Landuyt *et al.*, 2001 [74]
Radiation	Enhanced radiation damage when given before or after radiation in KHT tumors; enhanced radiation damage in C3 H tumors when given at the same time or after radiation	Murata *et al.*, 2001 [75]
Radiation	Decreased TCD_{50} when given 30–60 min after	Horsman and Murata 2002 [72]
Radioimmunotherapy (^{125}I-labeled anti-CEA antibody)	Combined treatment cured nude mice bearing colorectal tumors	Pedley *et al.*, 2001, 2002 [76,77]
Hyperthermia	Given 3 h before, increased BT4An rat glioma growth delay significantly compared with simultaneous administration of both agents	Eikesdal *et al.*, 2000, 2001 [78–80]
Hyperthermia	Enhanced C3 H mouse mammary tumor response when given 30 min after heat	Horsman *et al.*, 2000, 2002 [72,73]
Hyperthermia	Enhanced effects of hyperthermia in C3 H tumors	Murata *et al.*, 2001 [81]
Doxorubicin	Increased growth delay of a human medullary thyroid carcinoma model compared with doxorubicin alone	Nelkin and Ball, 2001 [68]
5-Fluorouracil	No significant MAC29 murine tumor growth delay with either agent alone, significant with combined treatment	Grosios *et al.*, 2000 [82]
Cisplatin	Significantly enhanced CaNT tumor growth delay when given pre- or post-cisplatin compared with effect of cisplatin alone	Chaplin *et al.*, 1999 [69]
Cisplatin	Additive tumor response when given 1 h after	Horsman *et al.*, 2000 [73]
Cisplatin	Increased cell killing in KHT, human breast (SKBR3) and ovarian (OW-1) tumor models when given 1–3 h after cisplatin	Siemann *et al.*, 2002 [83]
Cisplatin	Enhanced anti-tumor effects in a Kaposi's sarcoma model	Li *et al.*, 2002 [71]
Cisplatin	Increased cell killing in KHT, human breast (SKBR3) and ovarian (OW-1) tumor models; greatest effect when given 1 h after cisplatin	Siemann *et al.*, 2002 [83]
Cyclophosphamide	Increased cell killing in KHT, human breast (SKBR3) and ovarian (OW-1) tumor models when given 1 h after cyclophosphamide	Siemann *et al.*, 2002 [83]
Vinblastine	Enhanced anti-tumor effects in a Kaposi's sarcoma model	Li *et al.*, 2002 [71]
TNP-470	No increased growth delay in a rhabdomyosarcoma model	Landuyt *et al.*, 2001 [74]

TCD_{50} = radiation dose that controls 50% of tumors.

114 mg/m^2. The most common grade 1/2 toxicities were cardiovascular (tachycardia, bradycardia and hypertension). No QT_c prolongation was seen up to 4 h after CA4P administration. Tumor pain was common (35% of patients) and occurred a median of 40 min after the start of drug administration. Abdominal pain or cramping was also common, occurring in 24% of patients, and fatigue was experienced by 23% of patients, mainly at the higher drug doses and after repeated dosing. Visual toxicity was reported in 9% of patients. Twenty-six patients were evaluated for efficacy and a reduction in the mass of liver metastases of an adrenocortical carcinoma was measured using MRI. Pharmacokinetic samples from 27 patients showed a rapid dephosphorylation of CA4P to CA4 with a half-life of a few minutes followed by a slower metabolism to the glucuronide. Doses of 52–68 mg/m^2 were recommended for phase II studies.

Two other phase I studies have been reported in abstract form only. A phase I/pharmacokinetic trial is investigating CA4P administered as an i.v. bolus daily for 5 days every 21 days [50]. The drug was well tolerated and the MTD was 56 mg/m^2. A further phase Ib study examined CA4P in combination with carboplatin [51]. Patients received a 30-min infusion of carboplatin followed by a 10-min infusion of CA4P on day 1, with the schedule repeated every 3 weeks. The regimen was tolerated and reduced tumor blood flow 4–6 h following drug administration.

The clinical studies carried out to date show that CA4P when given in different schedules (weekly, 3-weekly, daily for 5 days every 21 days) is safe and lacks the hematological toxicity associated with many anti-cancer agents. Similar MTDs of around 50–60 mg/m^2 were found with three different dosing schedules. As pointed out elsewhere, the clinical data obtained to date with CA4P are similar to those for another class of tubulin-binding agents, the taxanes, in suggesting that the dosing schedule does not necessarily alter the range of total tolerable doses [48].

The importance of imaging

The rationale behind the investigation of functional imaging approaches for evaluating the *in vivo* effects of CA4P is to confirm the *in vivo* mechanism of action, assist the selection of doses for phase II trials and enable the selection of patients who are responding to treatment [52]. CA4P is active in experimental tumors at doses below the MTD. The MTD is used in phase I clinical trials to establish the dose to use for efficacy studies, and often stems from anti-proliferation/cytotoxic effects on, for example, the gut and hemopoietic cells. It was

thought that with CA4P tumor activity would be obtained well below the MTD. As the anti-vascular effects of CA4P can be measured as changes in tumor perfusion, this endpoint has been explored in preclinical and more recently clinical studies. A number of imaging approaches are being investigated [53], in particular magnetic resonance imaging (MRI) and positron emission tomography (PET).

MRI is the most widely investigated technique for imaging the *in vivo* effects of CA4P, in particular dynamic contrast enhanced-MRI (DCE-MRI). DCE-MRI involves the i.v. injection of the contrast agent gadolinium diethylenetriaminepentaacetate (Gd-DTPA). Gd-DTPA is a paramagnetic agent that decreases the longitudinal magnetization recovery time (T_1), thereby increasing signal intensity. The increase in contrast agent enhancement post- to pre-injection is proportional to its concentration in tissues. Gd-DTPA is poorly diffusible through normal tissue blood vessels, but escapes from leaky tumor vessels and is sometimes used in clinical practice to aid the delineation of tumors. Measurements of tumor enhancement are made over time (i.e. dynamic), and rapid and high levels of enhancement indicate permeable vessels, rapid blood perfusion and a large extracellular extravascular space. Different methods are used for the analysis of the data, both simple and kinetic. A widely used kinetic parameter is K^{trans}, which is thought to reflect tumor blood flow and vascular permeability, and V^e, thought to reflect the fractional volume of the extravascular extracellular space. Recommendations have been published for the appropriate methodologies to use in clinical trials of anti-angiogenic and anti-vascular agents [54]. K^{trans} or the initial area under the Gd-DTPA contrast agent time curve (IAUC) were recommended to be the primary end point. IAUC is a relatively simple non-modeled parameter [55].

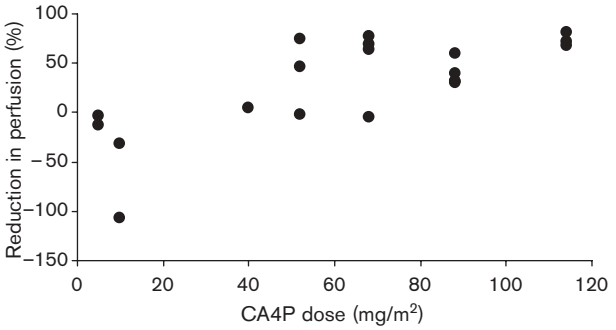
Preclinical studies showed the potential of DCE-MRI in measuring the anti-vascular effects of CA4P in tumors. Contrast enhancement was shown to decrease in experimental tumors following CA4P administration to tumor-bearing animals [45,56]. The differential susceptibility of a series of experimental tumors to CA4P was shown to correlate with prior MRI measurements of tumor vascular permeability [45]. Maxwell *et al.* [57] showed that the CA4P-induced changes in K^{trans} measured 6 h after the dosing of tumor-bearing rats were smaller in magnitude than changes in blood flow, although the time course and dose dependence were similar. Reductions in V^e were also measured. The authors showed that reductions in K^{trans} occurred uniformly across the tumors with no differences between the rim and center. Similar CA4P dose- and time-dependent effects were measured using IAUC. A variable response to CA4P has been reported using DCE-MRI [45,58]. In a study of two human colon adenocarcinoma xenografts, CA4P had little effect on one measured up to 3 h following drug administration [58]. Studies in cancer patients showed that the parameters V^e and IAUC were very reproducible and, although more variable, the parameter K^{trans} was also reproducible [55].

Imaging has been carried out as part of the phase I trials of CA4P. In the Dowlati *et al.* study [48] a reduction in tumor contrast agent enhancement was measured in six of seven patients treated at 60 mg/m^2 in the once-every-3-weeks study. In the weekly for 3 weeks followed by 1 week of rest schedule CA4P was shown to reduce K^{trans} in six of 16 patients treated at 52 mg/m^2 or above, with a significant mean reduction of 37 and 29% at 4 and 24 h, respectively [59]. The reductions in IAUC were 33 and 18%, respectively. No reductions were seen in muscle K^{trans} or kidney IAUC. The study showed a similar time course for the changes in rat and human tumors. In the daily for 5 days repeated every 3 weeks trial, reductions in K^{trans} in five of seven and in V^e in six of seven patients measured before treatment and 5–6 h following completion of the day 5 dose [60].

PET is also being explored for its potential to monitor the activity of CA4P in humans. PET methods for quantifying tissue perfusion using ^{15}O-labeled tracers are well validated, in particular the use of ^{15}O-labeled water ($H_2{}^{15}O$) [61,62]. The method has been shown to be reproducible in humans [63]. The results have recently been published from a $H_2{}^{15}O$ PET study that formed part of a phase I trial of CA4P [64]. PET measured CA4P-induced anti-vascular effects. Dose-dependent reductions in tumor perfusion were measured 30 min after CA4P administration with a mean reduction of 49% at a dose of 52 mg/m^2 or higher (Fig. 1). There was evidence of recovery 24 h following CA4P administration, but the reduction in tumor perfusion remained significant. Borderline significant effects were measured in normal tissues (spleen, kidney) with full recovery 24 h after CA4P dosing. In the same study PET measurements were also made of blood volume using $C^{15}O$. Significant CA4P-induced reductions in blood volume were measured 30 min following drug administration, which recovered by 24 h.

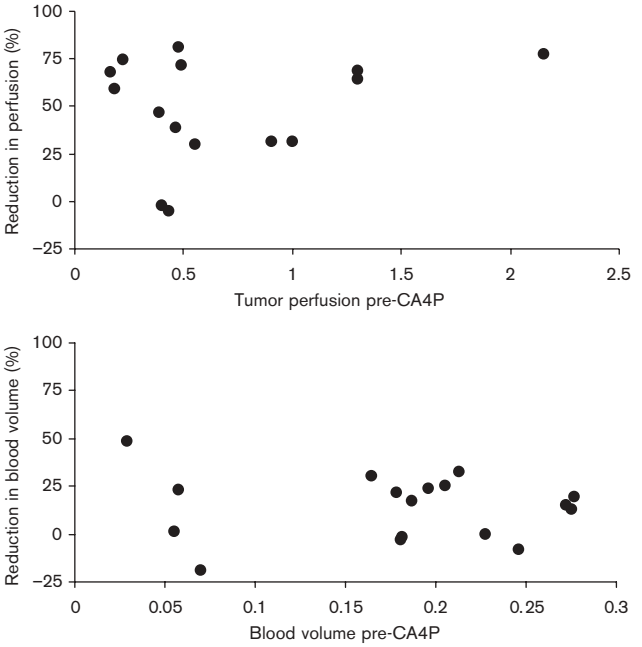
The imaging studies carried out to date in patients with advanced cancers have shown that human tumor perfusion is decreased in response to CA4P and that the effects are variable, with some tumors showing no drug-induced changes. Although anti-vascular effects are seen in normal tissues, compared with tumors they appear to be smaller and to recover more quickly. Although the imaging data were used for the selection of doses for phase II trials, there was little difference between the MTD and the dose at which anti-vascular effects were measured. A wide therapeutic index was predicted from animal

Fig. 1



CA4P dose-dependent changes in tumor perfusion measured using $H_2{}^{15}O$ PET 30 min following drug administration. Data from a series of patients with advanced cancers recruited in a phase I trial [64].

Fig. 2



Relationship between pretreatment tumor perfusion (top) or blood volume (bottom) and CA4P-induced changes in the vascular parameter. PET data from a series of patients with advanced cancers treated with 52–114 mg/m^2 CA4P in a phase I trial [64].

models, but in practice the toxicity seen was greater in man than in rodents [52]. The inter-patient variability in pretreatment data and in the changes measured in response to CA4P suggest that further studies are warranted to examine the pharmacodynamic potential of the imaging methods to select responding patients.

Future studies

Continuing studies of the mechanism of action should aid the development of second-generation drugs with increased tumor selectivity compared with CA4P and/or enable the optimization of the therapeutic index of CA4P. Increased understanding of the underlying mechanisms associated with anti-vascular effects on normal tissues will be useful. Understanding how transient the effects on normal tissues are and finding ways of minimizing them will both be important as well.

Determining the optimal dose and schedule will be another key development. The schedules examined in clinical trials so far have employed weekly, every 3 weeks and daily for 5 days dosing. The most impressive indication of efficacy was seen in the trial using an every-3-weeks dosing schedule [48]. However, the limited efficacy of CA4P towards experimental tumors when administered as a single dose was enhanced by using multiple dosing schedules. No significant growth delay was seen in tumors grown in mice given 500 mg/kg CA4P as a single dose, but a significant delay was observed when the same dose was given as 10 daily doses of 50 mg/kg [65]. A twice-daily dosing schedule (25 mg/kg twice a day) further increased growth delay compared with once-daily dosing [65]. Further support for the use of frequent dosing schedules as a method of improving therapeutic gain comes from the recovery in normal tissue perfusion measured using PET 24 h following dosing [52,64], and the short plasma half-life of CA4P and CA4 [48,49].

The optimum CA4P combination also needs to be established. Experimental data suggest that combinations with radiation and cisplatin are worthy of exploration in the clinic (Table 1), but there are other approaches that require investigation. An experimental tumor model resistant to CA4P was reported to have a high rate of nitric oxide production [46] and a value similar to that reported for some clinical samples [66,67]. Nitric oxide down-regulates neutrophil adhesion, may protect against a CA4P-induced neutrophil infiltration and/or may protect against cell death by oxygen starvation [67]. Enhancement of CA4P activity in experimental tumors was shown using nitric oxide synthase inhibitors [46,67].

Understanding the pharmacodynamic relationship will be another area of future research. Work in experimental tumors has shown that CA4P is more effective towards larger than small tumors [47]. It will be interesting to examine the effect of tumor size on response in human tumors. The relationship between perfusion status/vascularity and efficacy also needs to be explored in order to improve understanding of inter-tumor heterogeneity as a determinant of CA4P response. Imaging

studies should prove useful in increasing our understanding of the relationships between anti-vascular effects and pretreatment parameters, such as tumor size, perfusion and angiogenesis. These studies should help answer such questions as do well-perfused tumors respond better than poorly perfused tumors? Beauregard *et al.* [45] found a relationship between tumor vascular permeability and response to CA4P. Further support for the idea that tumors with better vascular parameters might respond between to CA4P comes from the dramatic response of a patient with a thyroid cancer (considered a vascular tumor) seen in one of the phase I trials [68]. However, an examination of PET data from a series of advanced human tumors suggests no relationship between pretreatment vascular parameters (perfusion or blood volume) and the level of CA4P-induced anti-vascular effects (Fig. 2). Clearly this is another area of interest for future studies.

Finally, there is some indication from experimental studies that there may be interest in a role for CA4P in the treatment of non-cancerous diseases with an angiogenic component [36,37].

Summary

There has been a clear drug development pathway for CA4P. Following the original proposal of the concept of vascular targeting and the search for drugs that selectively target the tumor vasculature, CA4P was selected for preclinical development. There has been the concurrent development of imaging methods for exploring anti-vascular effects *in vivo* and now promising data are emerging from the first clinical trials. Whether the current success will translate into a permanent role for the drug in the treatment of cancer remains to be established from ongoing phase II trials. The conclusion from this review of the published literature is that CA4P has considerable potential for improving the outcome of future cancer patients.

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