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REVIEW ARTICLE

COMBRETASTATIN DERIVATIVES IN CANCER: RECENT RESEARCH REVIEWED

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SUMMARY

Combretastatin is a vascular disrupting agent that has been very effective in disrupting tumor blood flow, resulting in enhanced tumor killing. Combretastatin may have detrimental effects on normal tissue, so analogues with the same properties as combretastatin are being developed in an effort to find the ideal drug that destroys all types of tumors while having no deleterious effects in normal tissue. This review focuses on a select few derivatives of combretastatin developed in the past few years that have shown some such promise. These drugs have passed quality tests in cell culture or in implanted tumor models. The compounds reviewed have been effective in causing various cancer cell lines to undergo apoptosis in culture, or in completely shutting down tumor blood flow in implanted tumor models. The majority of successful drug analogues have been more effective than combretastatin in either killing capacity or normal tissue sparing, or a combination of both. Some drugs have even resulted in complete tumor regression.

BACKGROUND

Cancer is a collection of diseases, and as such, it is very difficult to identify a successful strategy for treatment. The most common therapy regimen is to irradiate tissue and then administer a cytotoxic drug. This combinatorial approach to cancer therapy ideally results in killing of cancerous cells with the cytotoxic agent, followed closely by radiation damage of the normal tissue periphery, diminishing tumor regrowth; however, side effects are often quite severe. Another strategy is to target the only part of a tumor that is still physiologically normal, the endothelial cell, with antivascular compounds. Tumors are able to recruit endothelial cells and form a rudimentary vasculature sufficient to deliver blood, and therefore nutrients and oxygen, to the internal portions of the cancerous mass. Phenotypically, these endothelial cells are the same as newly formed vascular cells elsewhere in the body, and represent an ideal target. Tumors need vasculature to expand past the diffusion distance of oxygen, so blocking or diminishing the blood supply inhibits the ability of the tumor to thrive (grow and metastasize) (1, 2).

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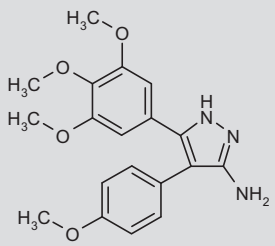
Combretastatin is one such antivascular compound. Isolated from a South African bush, *Combretum caffrum*, it is now referred to as combretastatin and is known to be an antimototic agent inhibiting tubulin polymerization (3). Tubulin polymerization is an essential step in structural integrity for newly formed endothelial cells; combretastatin causes a blebbing phenotype leading to constriction of blood flow. As the vasculature constricts, the tumor becomes more necrotic and has been shown to decrease in size. Petit et al. showed crude isolated fractions of combretastatin to have significant activity in the National Cancer Institute's in vitro astrocytoma assay, demonstrating 71-90% astrocyte reversal at relatively low concentrations (1-100 μg/mL) (4). These investigators went on to identify and purify several of the combretastatins, including A-1, B-1, B-3 and B-4 (5, 6), and the most potent tubulin inhibitor found was combretastatin A-4 (7). Combretastatin A-4 was tested in several murine leukemia and colon cancer cell lines and found to be the most antimitotic of the combretastatin constituents (7). Since that time, many analogues and derivatives of combretastatin have been used and this review will discuss the most recent work done with these antivascular compounds.

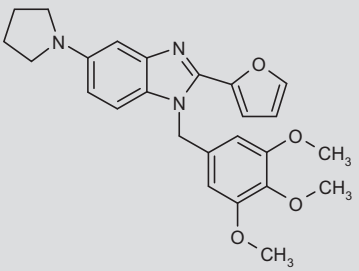
COMBRETASTATIN DERIVATIVES EFFECTIVE IN VITRO

XN-0502

The potency of combretastatin has been linked to the *cis*-orientation of the diaryl groups, and building on this information, Zhu et al. developed XN-0502 (8). Initial tests showed XN-0502 to be effective against a wide variety of human cancer cell lines, while exhibiting low toxicity in normal liver cells. XN-0502 exerts more robust antiproliferative effects against human non-small cell lung cancer (NSCLC) A549 cells than against normal human liver cells in vitro. XN-0502 disrupts microtubule formation in a concentration-dependent manner, and more severe destabilization was achieved with increasing concentrations. XN-0502 in increasing doses



XN-0502



FPTB

increased the populations of cells which arrested in the G_2/M phase of the cell cycle. Cell cycle progression stalled in the M phase due to incomplete microtubule condensation, at which point caspase-3 and caspase-9 were activated and poly[ADP-ribose]polymerase (PARP) cleavage was evident. The effectiveness of XN-0502 against tumor cells while sparing normal cells is a characteristic making this drug a potential target for further study.

ST-2151

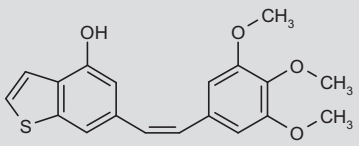
ST-2151 is a synthetic stilbenoid structurally related to combretastatin A-4. Vitale et al. tested the efficacy of ST-2151 compared to combretastatin A-4 in the treatment of NSCLC NCI-H460 cells in culture (9). For the study, investigators used concentrations in culture of twice the IC_{50} of the two drug compounds. Combretastatin A-4 more robustly inhibited tubulin polymerization and promoted apoptosis more strongly than ST-2151. Both compounds induced apoptosis through a caspase-dependent pathway. ST-2151, therefore, appears to be less effective as a cytotoxic agent then combretastatin A-4.

FPTB

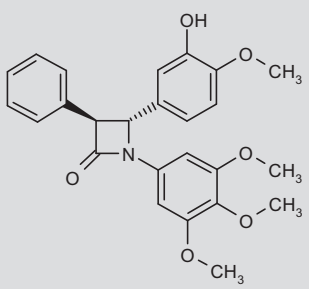
Chondrosarcoma is resistant to chemotherapy and radiation but responds well to FPTB therapy, while normal chondrocytes are spared (10, 11). FPTB induces cellular apoptosis through mitochondrial dysfunction, specifically decreasing the expression of Bcl-XL and Bcl-2, two proteins known to potentiate cancer cell survival. FPTB appeared to exert its apoptotic effects through increased levels of superoxide and hydrogen peroxide, an effect that was reversed with the use of antioxidants. FPTB also increases cleaved PARP, caspase-9 activity and the expression of cleaved caspase-12. The apoptotic effects of FPTB resulted from mitochondrial dysfunction and endoplasmic reticulum stress (10). Elevated reactive oxygen species (ROS) levels have long been associated with apoptosis in normal cells and it is interesting to note that the elevated levels were below the threshold to affect normal chondrocytes.

CA-432

Combretastatins can undergo *cis-trans* isomerization, which destabilizes the drug, leading to decreased efficacy and shelf life, and analogues have been created to address this issue. Plasma stability studies demonstrated that the insertion of a β-lactam ring prevents *cis-trans* isomerization, which provides up to 92% drug stability after 20 hours (12). Greene et al. tested a battery of combretastatin analogues against two cell lines, multidrug-resistant human chronic myelogenous leukemia K-562 cells and human myeloid leukemia HL-60 cells, which proliferate rapidly. Of the various compounds tested, CA-432 was shown to effectively stall cell progression in the G_2/M phase as early as 4 hours post-administration. CA-432 also induced phosphorylation of mitotic checkpoint serine/threonine-protein kinase BUB1 beta (hBUBR1) and Bcl-2 and Bcl-XL (both apoptosis-linked proteins), indicating microtubule disruption and leading to cell apoptosis. In the rapidly proliferating HL-60 cells, CA-432 induced a rapid apoptotic response, whereas in the multidrug-resistant K-562 cells the apoptosis occurred later. Both apoptotic responses were dependent on PARP cleavage and diminished procaspase 3 proteins. CA-432, as well as combretastatin, induced



ST-2151



CA-432

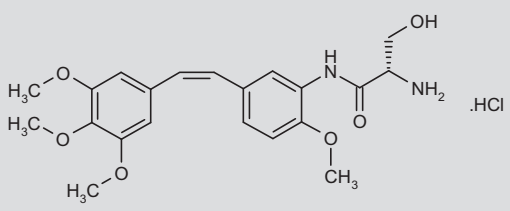
apoptosis in populations of tumors overexpressing phosphoglycolate phosphatase (PGP), a protein known to confer multidrug resistance. These data demonstrate the therapeutic efficacy of combretastatin and analogues over less effective chemotherapeutics in these settings. Another interesting characteristic of both combretastatin and CA-432 is their ability to induce apoptosis in cells with mutations in the *BCR-ABL* gene, a gene responsible for imatinib-resistant chronic myelogenous leukemia (CML).

COMBRETASTATIN DERIVATIVES EFFECTIVE IN VIVO

Ultimately, a successful drug will progress from cell culture to animal testing in either a spontaneous or implanted tumor system. Combretastatin is effective in disrupting tumor vasculature and can also be cytotoxic to normal tissue. Efforts have been made to bypass the negative side effects of combretastatin, such as by encapsulating the drug in a liposome and specifically targeting these liposomes to tumors using radiation therapy, which resulted in delay of tumor growth (13). Another approach to avoid side effects is to develop analogues of combretastatin and test their tumor reduction potency compared to combretastatin.

AC-7700 (ombrabulin hydrochloride)

Hepatocellular carcinomas are treated by surgical resection, although the recurrence rate is high, resulting in poor prognosis. Improvements in the identification of molecular markers indicating poor prognosis will go a long way to helping to develop treatment strategies for these tumors (14). The use of an antivascular drug nullifies the severity of tumors by disrupting the nutrient supply. AC-7700 (ombrabulin hydrochloride, AVE-8062), a derivative of combretastatin A-4, was used to treat hepatocellular carcinomas in rats (AH-130 cells) (15). Using in vivo microscopy, Ohno et al. were able to observe that 15 minutes following injection of AC-7700 into small-caliber vessels, those less than 20 μm in diameter began to disappear. Sixty minutes post-injection, all vessels except the largest, those over 30 μm in diameter, were completely undetectable, and in these large-caliber vessels flow was absent. At 15 minutes post-injection, vascular density was reduced by twofold. Treating the tumors with AC-7700 resulted in a 50% survival rate compared to the corresponding control animals. At 120 days post-injection, the endpoint of the study, there was no evidence of tumors or metastases in any of the surviving treated animals. AC-7700 treatment was also not associated with notable side effects, such as diarrhea, weight loss or anemia.



AC-7700

AC-7700 has also been successfully used in combination with cisplatin by Morinaga et al. in several tumor models (16). In the murine colon 26 tumor, this combination approach had a synergistic effect, which completely eradicated the presence of tumors. An added benefit of the combinatorial treatment was a greater accumulation of cisplatin within the tumor, without a concomitant increase in plasma or kidney levels. This combination approach was also effective in controlling the growth of implanted human colon carcinoma, human lung carcinoma, murine sarcoma and murine lung carcinoma tumors. This demonstrates the ability of AC-7700 to beneficially augment current therapies, contributing to the accumulation of chemotherapeutic agents in tumors while minimizing deposition in normal tissue.

Hori et al. used AC-7700 in combination with radiotherapy to reduce tumor blood flow (17). Implanted sarcomas responded to radiation therapy by increased tumor blood flow, peaking around 3 days post-irradiation, but interstitial pressure and tumor size progressively decreased. Intravital microscopy showed that AC-7700 therapy collapsed vascular perfusion within implanted sarcomas. Irradiating the tumor and waiting until blood flow reached a peak before administering AC-7700 resulted in a 50% cure rate in rats implanted with sarcomas.

In another study, Hori et al. tested AC-7700 to evaluate its ability to produce leaky vasculature in tumors (18). For this series of experiments, 50-nm micelles were used to determine tumor vasculature leakiness, and when tumors were less than 3 mm in diameter there was no accumulation of micelles. Irradiation alone showed no appreciable increases in the accumulation of these micelles. However, the addition of AC-7700 induced micelle accumulation within the tumor. These experiments demonstrated the ability of AC-7700 to damage tumor vasculature, increasing permeability, probably by extensive endothelial cell killing. Vascular leakiness caused by AC-7700 also helps to explain the accumulation of cisplatin in the combinatorial therapy shown by Morinaga et al. (16).

Hori et al. were also able to demonstrate that AC-7700 can constrict tumor-modified arterioles feeding implanted lung carcinoma tumors (19). For this study, the authors defined three groups of vasculature involved in perfusing carcinomas. The groups were as follows: feeding arterioles that provide the tumor with blood flow; tumor capillaries that provide nutrient exchange within the tumor; and drainage vessels that remove blood from the tumor. The effect of constricted feeding arterioles caused downstream collapse of tumor capillaries, some up to 50 μm in diameter, due to lack of blood flow pressure to maintain them. If the constriction of feeding arterioles was maintained for at least 2 hours, stagnant erythrocytes in the drainage vessels underwent hemolysis, contributing to bloodborne toxins. It is important to mention that the constriction of feeding arterioles was not observed using combretastatin A-4 phosphate (CA-4P) (20).

In yet another study by Hori et al., AC-7700 was shown to shut down tumor vasculature using Sato lung carcinoma cells implanted in rats (21). In this study, tissue blood flow was calculated using the hydrogen clearance method, giving a value for local blood flow. To determine perfusion within the tumor, fluorescent dye was injected and used in conjunction with an implanted tumor window. AC-7700 ther-

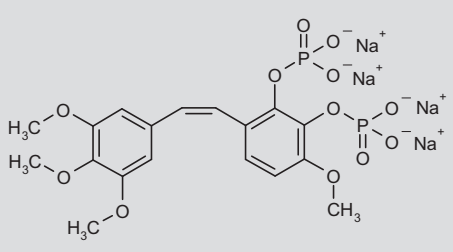
apy resulted in complete vessel shutdown, as evidenced by lack of fluorescent perfusion. The vessel shutdown was accompanied by a complete standstill in tumor volume changes. Histological analysis revealed drug damage primarily affecting tumor blood vessels. Hori et al. (22) continued to prove the efficacy of AC-7700 testing a wide variety of tumor locations. For this experiment, carcinoma or sarcoma cells were injected in the liver, stomach, kidney, muscle or lymph nodes. Following administration of AC-7700, liver tumor blood flow was almost completely abolished, whereas normal liver flow was only reduced by about 30-40%. Tumors located in the kidney, however, had complete vascular shutdown, with normal kidney blood flow remaining unaffected. In all instances, measured tumor blood flow was completely blocked between 30 and 60 minutes following injection of AC-7700 and was maintained for the duration of the observation (6 hours).

OXi-4503

Shaked et al. demonstrated that OXi-4503, a second-generation prodrug derivative of CA-4P, may contribute to tumor revascularization following use (23). Following radiation and cytotoxic treatment, there may be a viable rim of normal tissue remaining around the periphery of the tumor. This interface may contain tumor cells, and based on the research by Shaked et al., it may be stimulated to recruit bone marrow-derived circulating endothelial progenitor cells. OXi-4503 therapy stimulated circulating levels of stromal cell derived factor 1 (SDF-1), granulocyte colony-stimulating factor (G-CSF) and vascular endothelial growth factor (VEGF). The phenotypic changes leading to the enhanced recruitment of endothelial progenitor cells was dependent on G-CSF, identifying a potential target to disrupt in conjunction with the use of vascular disrupting agents. This study demonstrated a clear concern for anti-vascular compounds, or at least OXi-4503, in contributing to tumor regrowth following therapy.

DISCUSSION

Of the combretastatin derivatives mentioned in this review, only two were tested in vivo, OXi-4503 and AC-7700. Of these two, many more manuscripts have been published for AC-7700 detailing its efficacy in a variety of tumor models. The impact AC-7700 had on various implanted tumors highlights an extremely important consideration when dealing with vascular disrupting agents. Traditionally, for a tumor treatment strategy to be considered effective, the therapy must result in a marked decrease in tumor volume. As Hori et al. demonstrated, AC-7700 therapy resulted in vascular shutdown but no tumor volume change for up to 7 days post-treatment (21). The tumor volume did not decrease, however; arguably, the more important fact was that the tumor volume did not increase. During the same period of time, blood flow within the tumor dropped to 0 and did not deviate from there. Clearly, the drug is effective in stopping tumor blood flow, essentially starving the tumor. The problem is that, with the absence of blood flow, monocytes are unable to infiltrate the extravascular space and macrophages are unable to remove cellular debris. The stagnant blood flow results in a mass that will not decrease in volume for quite some time.



OXi-4503

Disruption of tumor blood flow is extremely effective in tumoricidal effects, although using tumor volume decrease as a primary indicator of efficacy is flawed. Tumors that decrease in volume following therapy may still have intact peripheral vasculature, allowing them to continue growing after the initial cytotoxic effects wear off. This vascularized periphery of a tumor is generally what radiotherapy is employed to disrupt. This is a region of physiologically normal tissue causing a vascular interface with the tumor, which can easily be reconnected to a tumor that has only been decreased in size. The only effective method is to completely disrupt this interface, and anti-vascular therapy is a perfect candidate, as was shown by Hori et al. Unfortunately, large-scale drug derivative analysis may pass over effective tumor-controlling drugs because they do not meet certain predefined criteria of tumor volume change.

Another problem with pipeline testing of combretastatin derivatives is that the majority are tested in cell culture on various tumor cell lines. Considering the fact that these derivatives are taking advantage of the vascular disruptive nature of combretastatin, the analysis of drug efficacy in tumor cell lines does not necessarily demonstrate their efficacy. Many of the cell culture tests measured the ability of the combretastatin derivative to disrupt tubulin binding, leading to apoptosis. This is useful information, as this is the mechanism by which combretastatin effects endothelial cell disruption. However, it is likely that many compounds deemed inefficient in tumor cell-killing properties may in fact be extremely effective in disrupting tumor blood flow. ST-2151 is a compound that was able to disrupt tubulin binding in tumor cells, but was not as effective as combretastatin (9). This drug may be more effective than combretastatin in disrupting tumor blood flow and sparing normal tissue. However, these tests may never be performed because of its lower efficacy compared to combretastatin in disrupting tumor cells. Overall, in the past few years, several analogues of combretastatin have been developed that show good efficacy in disrupting microtubule condensation and activating apoptosis pathways, and several have advanced to clinical trials.

Currently, two of the compounds reported in this review are undergoing clinical trials. OXi-4503, developed by OXiGENE, is being evaluated in three clinical trials, one of which has been completed, another that is ongoing and another that is slated to start soon. The recently completed study was a phase I project using OXi-4503 in solid tumors to determine its maximum tolerated dose with weekly i.v. infusions (ClinicalTrials.gov Identifier: NCT00977210). The ongoing trial is a phase Ib/II study which will determine the safety and efficacy of OXi-4503 in patients with primary or secondary hepatic tumors

(ClinicalTrials.gov Identifier: NCT00960557). The third trial is a phase I study in the recruitment stage and will be looking at the efficacy of OXi-4503 in treating relapsed and refractory acute myelogenous leukemia and myelodysplastic syndromes (ClinicalTrials.gov Identifier: NCT01085656). All of these trials are using OXi-4503 as a stand-alone drug approach to treating solid tumors.

AC-7700 is the other compound mentioned in this review that is currently undergoing clinical trials. AC-7700 is in development by sanofi-aventis and is currently involved in nine clinical trials. One of these studies was recently completed and was a phase I trial using AC-7700 in combination with chemotherapy for the treatment of neoplasms (ClinicalTrials.gov Identifier: NCT00719524). Another phase I trial currently ongoing is using a carbon-14-conjugated form of AC-7700 to investigate systemic distribution and radioactivity in treating malignant neoplasms (ClinicalTrials.gov Identifier: NCT01063946). To determine the dose-limiting concentration of AC-7700, a dose-escalating phase I trial is being performed in patients with solid tumors (ClinicalTrials.gov Identifier: NCT00968916). A phase II/III trial in soft tissue sarcoma involves the use of AC-7700 in combination with cisplatin to determine progression-free survival (ClinicalTrials.gov Identifier: NCT00699517). Sanofi-aventis is also launching a phase I trial to determine the maximum tolerated doses of AC-7700 and bevacizumab as combination therapy for malignant neoplasms (ClinicalTrials.gov Identifier: NCT01193595). Yet another phase I trial will determine the efficacy of AC-7700 and cisplatin in malignant neoplasms, with treatments administered every 3 weeks (ClinicalTrials.gov Identifier: NCT01021150). Sanofi-aventis is recruiting for another phase I study that will combine AC-7700, cisplatin and docetaxel as 3-week injections administered to patients with malignant neoplasms (ClinicalTrials.gov Identifier: NCT01095302). A phase II trial is also recruiting patients with NSCLC to evaluate progression-free survival upon treatment with AC-7700, taxane and platinum (ClinicalTrials.gov Identifier: NCT01263886). Finally, the last phase I trial sanofi-aventis is recruiting for will involve the treatment of advanced solid tumors with a combinatorial approach using AC-7700, paclitaxel and carboplatin (ClinicalTrials.gov Identifier: NCT01293630). Just as there is considerably more literature available discussing the potential success of AC-7700, there are also many more clinical trials ongoing and recruiting than for OXi-4503.

Sanofi-aventis appears to be promoting AC-7700 as a therapy for combination with other chemotherapeutic agents. It will be interesting to follow these trials to see if AC-7700 is able to reduce the side effects of the chemotherapeutic agents used in conjunction. One would expect a trapping of the chemotherapeutic agent within the tumor, as the literature identifies AC-7700 as being extremely effective in rapidly collapsing tumor vasculature. However, I believe it will be of equal interest to monitor the progress of OXi-4503 in clinical trials as a stand-alone agent. A wider variety of anti-vascular agents will greatly benefit patients with tumors. The number of clinical trials these agents are involved in is very promising.

ACKNOWLEDGMENTS

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DISCLOSURES

The author states no conflicts of interest.

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