Cancer Letters 403 (2017) 289–295

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Suppression of angiogenesis and tumour progression by combretastatin and derivatives

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ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form
10 June 2017
Accepted 28 June 2017

Keywords:
Angiogenesis inhibitor
Cell motility and invasion
Cell proliferation
Combretastatins
Suppression of EMT
Suppression of tumour progression

ABSTRACT

The search for small molecule inhibitors has gained prominence with the recognition of their inherent advantage for cancer therapy. Combretastatin is a naturally occurring small stilbenoid. By virtue of the ability to bind to tubulin combretastatin and its derivatives promote depolymerisation of microtubules as well as inhibit tubulin polymerisation. This suppresses cell proliferation signalling and induces apoptosis. Combretastatins activate mitotic checkpoints that lead to mitotic catastrophe and apoptosis. They subvert the signalling systems which stimulate invasion, activate EMT (epithelial mesenchyme transition) and promote tumour progression. Allied with the ability to suppress angiogenesis these compounds have been viewed as potential inhibitors of metastasis.

The notion of merging RTK (receptor tyrosine kinase) inhibition with suppression of invasion and possible inhibition of EMT has contributed to the credibility of combretastatins as anti-cancer agents. Invaluable are their attributes of inhibiting tumour growth and induction of apoptosis and necrosis by reducing blood supply to the tumour. Aside from these biological effects, this commentary also discusses the issues of the targeting of combretastatins to the tumour vasculature and effective delivery of the drugs encapsulated in nanospheres. Notwithstanding the perceived benefits, one can see a compelling need to understand the effects of combretastatin on the actin cytoskeletal dynamics and the disruption of microtubule polymerisation, and whether it is more efficient a tumour inhibitor than the conventional drugs that target microtubule dynamics. Combinations of combretastatins with other vascular disrupting agents have been attempted. It is essential to establish the perceived inhibition of EMT beyond reasonable doubt. This might justify using the combretastatins with allosteric EMT and Akt inhibitors as additional choices for pre-clinical/clinical studies.

The stilbenoids are a family of polyphenols which are biologically active derivatives of stilbene. They are naturally occurring compounds of plant origin. Stilbenoids have been called phytoalexins. Plants synthesise phytoalexins as a response to environmental stresses and fungal, viral and bacterial infection [1]. Most prominent are the products of Vitaceae family grape vine *Vitis vinifera*, e.g. resveratrol, a highly efficient anti-cancer agent. Combretastatin (combretastatin A4) (molecular mass, 396.32 g/mol) is a naturally occurring small stilbenoid derived from the African Bushwillow *Combretum caffrum*. The discovery, development and the cytotoxic properties of combretastatin and its analogues were reported many years ago [2–4]. The combretastatins show geometric isomerisation that can occur starting with both *cis*- and *trans*-isomers by photo-activation or in response to exposure to acidic media. The photo-activation of cis↔trans isomerisation and the mechanism involved in the process have been described [5]. The *cis*-isomer does isomerise over time into the more stable *trans*-isoform. Combretastatin is more active in the cis configuration than in the *trans*-isomer form. The isomeric stability and activity are the reason why much effort has been directed to the development of derivative restricted to the *cis*-geometry [6]. The combretastatins are poorly soluble in water. Combretastatin phosphate is a highly water soluble pro-drug developed some year ago [7,8]. It is dephosphorylated and rapidly internalised by tumour tissues [9]. Furthermore, the *cis* isomer binds to tubulin with several magnitudes greater affinity than the *trans* isomer. This could explain the differences in the potency of the two isoforms [10]. With this

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http://dx.doi.org/10.1016/j.canlet.2017.06.032
0304-3835/

perspective, this review is restricted by design to a discussion of the phenotypic effects of combretastatin, its analogues and related stilbenoids on cancer. The actual development of the drug, its isolation, characterisation and the evolution of its analogues are not within the purview here.

The search for small molecule inhibitors (<900–1000 Da) of the processes accompanying or triggering tumour growth and progression has assumed primacy in recent years, given the recognition of the advantages inherent in the use of small molecules in cancer therapy. An important consideration in the design of new drugs is permeability which is dictated by the molecular size and structure of the putative candidate. Lower molecular mass is conducive to cell permeation. Permeability is governed by the diffusion constant across the membrane, the thickness of the membrane and the partition coefficient of the drug [11]. Higher the partition coefficient the more lipophilic and permeable the drug would be and also less liable to efflux from the cells. Small molecule drugs are non-immunogenic and tend to be stable. Also, small molecules and peptides are likely to be well tolerated. This profile of features has to be balanced with efficacy in therapy. Targeted delivery of the drugs is crucial in treatment. The nanoparticle technology can overcome the drug solubility impediments and also aid more efficient delivery of the drugs to their targets by tagging monoclonal antibodies to the nanoparticles with the encapsulated drug. The nanoparticles are usually copolymers of lipophilic molecules and therefore would enable the delivery of relatively non-lipophilic drugs to the targets.

There have been consistent efforts over the past few years to develop inhibitors with small molecular mass which suppress growth factor receptor signalling, and the signalling systems controlling the processes of invasion and motility and the activation of EMT (epithelial mesenchyme transition) of cancer cells. The identification of small molecule drugs and the designing and development of novel ones to suppress angiogenesis have not lagged behind. Both cell membrane-bound and nuclear receptors of ligands that can alter biological response have been the major foci of allosteric mode of signalling. Allosteric modulation involves conformational changes in a receptor upon binding by its natural allosteric ligand to generate an altered signal output. In this case the altered output in the form of the biological effect may occur due to the emergence of new but distant binding sites that can engage non-canonical ligands. The phenotypic output of allosteric alterations can also result from changes in the affinity of the binding of the canonical ligand to the primary binding site. The concept of molecular reorganisation has been deployed for developing allosteric drugs [12,13]. Some of them have been viewed as prime candidates for the inhibition of angiogenesis [14]. The notion of merging inhibition of growth by RTK (receptor tyrosine kinase) suppression with concurrent constraint on invasion, and impeding EMT and the development and survival of CSCs (cancer stem cells) has gained much credibility. These are an integral ingredient of tumour progression. The mTOR (mammalian target of rapamycin) pathway co-ordinates signalling systems that incorporate growth factor receptor signalling, EMT activation and angiogenic signalling [15,16]. It integrates signal transduction by many RTKs, including RTKs of angiogenic activators, and the PI3K/Akt/NF-κB axis. This concept has been expounded in detail recently. This integrative function has provided powerful impetus to achieve allosteric modulation of mTOR signalling to influence cell proliferation, EMT and angiogenesis. Several inhibitors of the intermediaries of angiogenic signalling, angiogenic growth factor RTKs and allosteric inhibitors of angiogenesis have been identified. Besides these, of note are phytochemicals that have proffered much promise to combat angiogenesis and control tumour growth and possibly also metastatic spread [14].

Mode of action of combretastatins

The targeting of microtubule dynamics has evolved steadily over the years yielding many anticancer agents that stabilise or destabilise the microtubule system. There are two domains in tubulin to which these drugs bind. They are the vinca binding domain and the colchicine domain. Colchicine, combretastatin and podophyllotoxin are colchicine domain binding drugs [17]. Vincristine and vinblastine are vinca domain binding drugs, as the name would suggest. There are also semi-synthetic derivatives of these first generation drugs [18]. The taxane binding site to which paclitaxel, docetaxel, and related molecule bind many not be totally distinct from the colchicine site. Two taxane domains have been identified, which are not too distant from each other. Interestingly, these could be a part of the colchicine domain [19,20], albeit taxanes and colchicine binding drugs work in the opposite direction in respect of tubulin polymerisation.

There is no obvious competition between the agents that bind either the vinca or the colchicine domain. The polymerisation of tubulin requires the interacting heterodimers to be in the straight configuration. With the binding of drugs the pro-filaments remain curved. Colchicine binding prevents the tubulin subunits reverting to the straight from the curved conformation and this suppresses the assembly of microtubules [21]. Combretastatin disrupts microtubule assembly by binding to the colchicine binding site [22,23]. The colchicine binding pocket has a major role in tubulin polymerisation, so much so, many drugs that bind to the colchicine pocket has been adduced as inhibitors of tubulin polymerisation [24]. The efficacy of combretastatin derivatives corresponds closely with the affinity of their binding to tubulin [25]. In common with other tubulin disrupting agents, combretastatin depolymerises tubulin assembly and by interfering in this dynamics, it is able to arrest the progression of the cell cycle at the G2/M phase. This occurs in consort with the activation of pathways that signal apoptosis [26]. The disruption of tubulin dynamics can result in abnormal or early entry into mitosis and lead to mitotic catastrophe.

Signalling systems suppressed by combretastatins

The combretastatins downregulate the major signalling systems which promote angiogenesis, cell proliferation and invasion. A valuable attribute is inhibiting tumour growth with the induction of apoptosis and necrosis by reducing blood supply. The potential value is further emphasised by the demonstration that they can inhibit cell proliferation as well as suppress cell motility. These traits underscore the versatility of combretastatins.

Given the prominent role played by angiogenesis in tumour dissemination the suppression of the metastatic process by combretastatins has also been actively pursued. This article represents a brief commentary dedicated to discussing the salient aspects of combretastatin function as relevant to the development and progression of cancer and assessing the potential of the stilbenoids as anticancer agents and exploring the signalling systems that might provide clues to the potentiation of their effects (Fig. 1).

Effects of combretastatin on cell proliferation signalling

Many analogues or derivatives of combretastatin have been synthesised. A majority of the compounds have shown the propensity to inhibit cell cycle progression at the G2/M interphase. Lin et al. [27] suggested some while ago that combretastatin suppressed invasion and cell proliferation by inhibiting PI3K/Akt signalling. The stilbenoid was effective only in the cell line that constitutively displayed Akt activation. They also found that the