Screening For Antiangiogenesis Activity In Natural Products: A Review

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Article ID: WMC001315

Article Type: Review articles

Submitted on: 10-Dec-2010, 03:23:01 PM GMT    Published on: 11-Dec-2010, 02:15:29 PM GMT

Article URL: http://www.webmedcentral.com/article_view/1315

Subject Categories: PHARMACOLOGY

Keywords: Angiogenesis, Screening models, Rat aortic rings, Endothelial cells, Medicinal plants.

How to cite the article: Aisha A, M. Abu-Salah K, Ismail Z, Abdul Majid A. Screening For Antiangiogenesis Activity In Natural Products: A Review . WebmedCentral PHARMACOLOGY 2010;1(12):WMC001315

Source(s) of Funding: University of Science Malaysia

Competing Interests: No competing interests exist
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Abstract

Angiogenesis is a process of new blood vessel development that plays a vital role in embryonic development and numerous pathological conditions including cancer, rheumatoid arthritis, obesity, diabetic retinopathy, age related macular degeneration (AMD) and neurological disorders such as Parkinson and Alzheimer Disease. Recently a number of agents that inhibit angiogenesis have been approved to treat diseases such as cancer and AMD. The potential use of natural products to target angiogenesis is beginning to be appreciated especially their role in chemoprevention. This review will address some of the technical aspects in screening for antiangiogenic activity in natural products.

Keywords: Angiogenesis, Screening models, Rat aortic rings, Endothelial cells, Medicinal plants.

Introduction

Angiogenesis: definition and consequences

Angiogenesis, the formation of new blood vessels is a biological process that plays a fundamental role in embryonic development. In adults, angiogenesis plays an important role in some physiological situations such as in the female reproductive tract, in the placenta during pregnancy, and during wound healing (Auerbach et al., 2003; Folkman, 1995; Vailhe, Vittet & Feige, 2001). Pathological angiogenesis is unregulated process believed to play a key role in several pathological conditions including proliferative retinopathies, atherosclerosis, rheumatoid arthritis, psoriasis and tumor growth and metastasis (Creamer et al., 2002; Folkman, 1990; Folkman, 1995). Since tumor angiogenesis is essential for growth and metastasis of most solid malignancies (Folkman, 1990; Folkman, 1995) it has became a main target for developing novel cancer therapies (Eatoch, Schatzlein & Kaye, 2000; Hurwitz et al., 2004; Pfeffer et al., 2003; Scappaticci, 2003). In this context this study was undertaken to investigate the antiangiogenic potential of herbal extracts aiming to develop new and effective anticancer therapies.

Angiogenesis: the sequence of events

To develop effective angiogenesis inhibitors, it is crucial to understand the mechanisms underlying the process. In tumor microenvironment, angiogenesis occurs as a consequence of angiogenic imbalance in which the proangiogenic factors predominate over antiangiogenic factors (Giordano & Johnson, 2001; Udagawa et al., 2002). The principal cells involved are endothelial cells, which form the lining of blood vessels and constitute almost the entirety of capillaries (Auerbach et al., 2003). Angiogenesis starts in response to stimulation by proangiogenic factors secreted by different types of cells including tumor cells, activated lymphocytes, or wound-associated macrophages causing activation of endothelial cells (Auerbach et al., 2003; Nicosia & Ottinetti, 1990; Risau, 1990). To form new blood vessels, the activated endothelial cells must first escape from their original location through the blood vessel’s basement membrane and its surrounding interstitial fluid, and then migrate towards the source of stimulus. Behind the migrating front, endothelial cells proliferate to provide the required number of cells that will reorganize to form three dimensional tubular structures. The final phase and the includes arrest of endothelial cells proliferation and stabilization of the immature capillary with pericytes and basement membrane (Auerbach et al., 2003; Benjamin, Hemo & Keshet, 1998; Bergers & Song, 2005; Vailhe, Vittet & Feige, 2001). Each step of this process can be a target for intervention by angiogenic modulators.

In vitro angiogenesis models

The main practical challenge in angiogenesis studies is the selection of the right model. An ideal model would take into account all the representative steps of angiogenesis, from detachment of endothelial cells...
from the vascular wall to the final tubular morphogenesis, its maturation, and connection to a functional vascular network (Vailhe, Vittet & Feige, 2001). Furthermore, the model should be robust, rapid, reproducible, and easily quantifiable and it should allow assessment of multi-parameters including positive and negative controls (Staton, Reed & Brown, 2009; Vailhe, Vittet & Feige, 2001). Despite the availability of several in vitro models of angiogenesis, there still no standard model that can address all the steps occur in neovascularization. Therefore, a combination of different models is essential to cover the entire range of events during angiogenesis (Staton, Reed & Brown, 2009; Staton et al., 2004).

In vitro angiogenesis models include assays that utilize either cultured endothelial cells or tissue explants. Assays utilizing cultured endothelial cells include cell proliferation, migration, invasion and differentiation (Auerbach et al., 2003; Staton, Reed & Brown, 2009). These assays are robust, reproducible and applicable for high throughput screening (Arnaoutova & Kleinman, 2010; Liang, Park & Guan, 2007; Mosmann, 1983). However they have some drawbacks. In vitro assays involve the use of cultured endothelial cells which are propagated in a single culture. In addition the cells are in proliferation state which may interfere with the test compounds especially when targeting proangiogenic factors. On the contrary, in vivo angiogenesis process involves not only the quiescent endothelial cells, but involves interaction with other cell types including pericytes, smooth muscle cells, fibroblasts, macrophages and tumor cells. In addition, endothelial cells are heterogenic since there are microvascular and macrovascular cells which differ in their morphology, physiology and response to angiogenesis modulating agents (Staton, Reed & Brown, 2009; Staton et al., 2004; Vailhe, Vittet & Feige, 2001). More important, passing endothelial cells in vitro is associated with loss of their normal physiological properties, and resulting in variations in the results which may affect reproducibility of the model (Staton, Reed & Brown, 2009). Despite these limitations, the in vitro angiogenesis models that utilize endothelial cells still can be used but in combination with other in vivo or ex-vivo models in high throughput screening, and to get clues about the mechanism of action of pro- or anti-angiogenic agents by targeting particular events of angiogenesis (Auerbach et al., 2003). However care should be taken to select the right type of endothelial cells and to use cells at low passage number (Staton, Reed & Brown, 2009).

The other category of in vitro angiogenesis assays is based on the ability of activated endothelial cells to invade three dimensional substrate (Vailhe, Vittet & Feige, 2001), or the ability of tissue explants embedded within the substrate to form microvessels. Examples of tissue explants include rat or mouse aortic rings, porcine carotid artery, chick aortic arch, placental vein disk and fetal mouse bone explant (Nicosia & Ottinetti, 1990; Staton et al., 2004; Vailhe, Vittet & Feige, 2001). In this study we used a combination of the rat aortic rings as the primary screening model and endothelial cell proliferation in order to get the maximum benefits from these in vitro tests.

Rat aortic rings

The rat aortic rings model of angiogenesis was first developed by Nicosia and Ottinetti (Nicosia & Ottinetti, 1990). In this model, rat aortic rings are embedded in a matrix such as collagen, or fibrin gel and cultured in an optimized serum-free medium. A complex network of branching microvessels develops from endothelial cells of aortic intima in response to endogenous growth factors released from the dissected aortas (Nicosia et al., 1997). Quantification is can be achieved by measuring the number, length, or the area of microvessels outgrowth from the primary aortic explants (Brown et al., 1996; Nicosia et al., 1997).

The rat aortic rings is the most used angiogenesis model (Auerbach et al., 2003), and considered by many researchers to essentially simulate the in vivo angiogenesis environment since it involves the surrounding nonendothelial cells such as smooth muscle cells, pericytes and a supporting matrix (Bergers & Song, 2005; Hall, 2006; Howson et al., 2005; Nicosia & Villaschi, 1995). In addition, the endothelial cells are not altered by repeated passaging and are quiescent at the time of explantation and consequently are more representative of the situation found in vivo where angiogenesis is triggered and quiescent endothelial cells respond by becoming proliferative, migrating out from the existing vessels and differentiating into tubules (Staton, Reed & Brown, 2009; Staton et al., 2004). External growth factor supplements are not required since the required growth factors including VEGF are provided endogenously from the dissected aortas (Nicosia et al., 1997), and by a subset of immature immunocytes that can proliferate and differentiate into macrophages and ultimately stimulating angiogenesis (Zorzi et al.,
Other advantage of organ explant models of angiogenesis include the low cost, easy manipulation of treatment conditions, lack of inflammatory response seen with in vivo models and the possibility of generating many aortic rings from one animal (Kruger et al., 2001).

A major problem with all organ culture models of angiogenesis is the use of non-human tissues, which questions their applicability as preclinical screening assays since responses may be species specific (Staton, Reed & Brown, 2009). Even though, rat aortic model is a representative of almost all steps of angiogenesis except the blood flow, the model is not fully representative of the microvascular environment encountered in some diseases such as tumor microenvironment (Auerbach et al. 2000). Another drawback of these models arise during interpretation of results especially when looking for antiangiogenic agents since the inhibition of microvessels outgrowth may be due to nonselective cytotoxic activity rather than due to a ‘true’ antiangiogenic effect. Overall, the rat aortic model of angiogenesis is still the best in vitro model because it mimics the in vivo environment of angiogenesis in terms of initiation and the cascade of events; however other in vitro and in vivo models are needed to further support and confirm the results and to exclude species specific response.

**Proliferation of endothelial cells model**

Inhibition of the microvessels outgrowth from aortic rings may be due to nonselective cytotoxic effect induced by the test compounds or due to ‘true’ antiangiogenic activity. In order to discriminate the nonselective cytotoxic from antiangiogenic effects, extracts with more than 50% inhibition in rat aortic rings should be evaluated for cytotoxicity on human umbilical vein endothelial cells (HUVECs) and other human cell lines. The main interest is to find extracts with significant inhibition of the microvessels outgrowth in rat aortic rings, without cytotoxic effect on HUVECs, or the extracts should be selective cytotoxic towards HUVECs. Besides distinguishing extracts with antiangiogenic activity from those with nonselective cytotoxicity, the combination of these two models can help to get insights into the mechanism of action of extracts with interesting activity.

**Medicinal plants as a source of antiangiogenic agents**

Medicinal plants continue to provide new and important leads against different pharmacological targets including cancer, AIDS, Alzheimer’s disease, malaria, and pain (Balunas & Kinghorn, 2005). Inhibition of angiogenesis which was suggested by Folkman on 1971 is now considered to be one of the most promising strategies leading to the development of new antineoplastic therapies (Folkman, 1971). Accordingly, numerous bioactive plant-derived compounds have been tested for their antiangiogenic potential. Among the most frequently studied are polyphenols present in fruits and vegetables (Cao, Cao & Brakenhielm, 2002; Mojzis et al., 2008). There are close to 5000 different polyphenols described so far which are divided into subgroups including isoflavones, flavonoids and lignans (Cao, Cao & Brakenhielm, 2002). Recently, several polyphenols isolated from various plants have been found to be potent inhibitors of angiogenesis for example catechins from green tea (Leong, Mathur & Greene, 2009), resveratrol from grapes and other sources (Cao et al., 2005), quercetin (Tan et al., 2003), rosmarinic acid (Huang & Zheng, 2006), genistein (Su et al., 2005), curcumin (Arbiser et al., 1998) and several other compounds. Other compounds were also reported with interesting antiangiogenic activity including the triterpenes such as ursolic acid (Kanjoormana & Kuttan, 2010), oleanolic acid (Sogno et al., 2009), lupeol (You et al., 2003) and betulinic acid (Mukherjee et al., 2004). Polyphenols and triterpenes are among the most abundant secondary metabolites in higher plants (Jager et al., 2009; Mojzis et al., 2008), therefore medicinal plants can provide high potentiality for discovery of new and effective antiangiogenic agents.

**Criteria for plants selection**

Generally plants those are rich in polyphenols, triterpenes with strong antioxidant and anti-inflammatory effects tend to have antiangiogenic activity. Polyphenols and triterpenes have a wide range of pharmacological activities including antioxidant, anti-inflammatory, cardioprotective, and anticancer (Liu, 1995; Mojzis et al., 2008). Other compounds that have anti-inflammatory and antioxidant activity with good antiangiogenic activity includes betulinic acid, green tea catechins, vitamin E and resveratrol (Cao, Cao & Brakenhielm, 2002; Huang & Zheng, 2006; Mojzis et al., 2008; Mukherjee et al., 2004; Tan et al., 2003). Amongst the many...
plants with good antiangiogenic activity include *Punica granatum* L (Toi et al., 2003). *Garcinia mangostana* L which have been shown to have strong antioxidant and anti-inflammatory effects could good source of new and effective antiangiogenic agents (Chen, Yang & Wang, 2008; Moongkarndi et al., 2004). Rasadah and his colleagues wrote on the anti-inflammatory activity of *Sandoricum koetjape* Merr (Rasadah et al., 2004). Anti-inflammatory activity of Syzygium species from the family Myrtaceae has also been reported (Muruganandan et al., 2001). *Delonix regia* was found to be rich in carotinoids with a well known antioxidant activity (Jungalwala & Cama, 1962). Extracts from *Cassia fistula* Linn were reported to have both antioxidant and anti-inflammatory effects (Ilavarasana, Mallikab & Venkataramanc, 2005).

All in all, many natural products tend to have high level of antioxidant property, a key feature that is useful in angiogenesis inhibition. It is thus not surprising to find many of them can potentially prevent cancer growth and development given the vital role of angiogenesis in cancer. It is however, important to utilize effective screening methods that can detect anti-angiogenic activity via the various routes and pathways. Without such methods, potential lead compounds may be missed.

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