Review

Shark Cartilage as Source of Antiangiogenic Compounds: From Basic to Clinical Research

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The discovery that angiogenesis is a key condition for the growth of a tumor beyond a millimeter or two, brings about a new approach in the treatment of tumors using drugs able to inhibit the formation of new blood vessels. Also, it has been realized that antiangiogenic drugs can be useful in the treatment of other pathological processes, now classified as angiogenesis-dependent diseases. Initially, cartilage was considered as a possible natural source of antiangiogenic compounds due to its known avascular nature. To date, a number of *in vitro* and *in vivo* studies have suggested the existence of antiangiogenic and antitumor compounds in bovine and shark cartilage. However, the potential usefulness of shark cartilage in the treatment of cancer and other angiogenesis-dependent diseases have not been totally accepted due to (i) unsatisfactory patient outcome in clinical trials that have used shark cartilage in cancer patients, (ii) the lack of data that correlates bioavailability with pharmacological effects using oral shark cartilage. Thus, the objective of this review is to describe the main basic and clinical investigations reported in the literature, in which the antiangiogenic and/or antitumor properties of shark cartilage or of its extracts were evaluated. Possible explanations for conflicting results are discussed as well.

Key words shark cartilage; angiogenesis; cancer

The concept that the growth of a tumor depends on new vascularization was first proposed by Judah Folkman. His historic and classical hypothesis was first published in 1972 and later expressed as: "once tumor take has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor."[1,2] This idea led to a new and promising direction in the field of cancer treatment based on the logical reasoning that the inhibition of new vascularization induced by tumors would lead to arrest tumor growth. Moreover, not only cancers but also some inflammatory conditions such as arthritis and psoriasis as well as metabolic diseases including diabetic retinopathy, currently viewed as angiogenesis-dependent diseases, are a major focus of attention because the inhibition of angiogenesis could be a new approach in their treatment as well.[3]

The concept of an antiangiogenesis approach as a promising anticancer strategy led to the discovery of many natural and synthetic compounds with anti-angiogenic properties.[4] Cartilage was the first normal tissue to be regarded as a natural source of antiangiogenic compounds, principally because cartilage is a normally avascular tissue. Additionally, some clinical evidence pointed to that possibility; for instance, bone tumors such as osteogenic sarcoma do not spread to adjacent cartilage and cartilage of the spinal column rarely is affected when breast cancer metastasizes to the vertebrae. Further, chondrosarcoma, a tumor of cartilage tissue, is the least vascularized of all solid tumors.[5,6]

Initially, cartilage was obtained from calves to test for its ability to inhibit the development of neovascularization. However, Lee and coworkers realized that calves had only a small quantity of cartilage that represented scarcely 0.6% of the total body weight in this animal. Thus, it was decided to use sharks as a source of cartilage because the shark endoskeleton is entirely formed by cartilage representing 6—8% of the total body weight.[7] An additional interest in sharks as a source of therapeutic compounds arose from the observation that in general sharks do not get cancer.[8,9]

The efficacy of shark cartilage and derived compounds in the treatment of several angiogenesis-dependent diseases, especially in cancer, has been a controversial issue over the last ten years. Thus, the purpose of this review is to summarize what has been published in this respect and to weigh the evidence for and against the potential utility of shark cartilage preparations in the treatment of cancer.

Antiangiogenic and Antitumor Activities of Cartilage-Derived Preparations To date, there have been numerous studies supporting the hypothesis that cartilage is a source of antiangiogenic and antitumor compounds. The first experimental study to examine the antiangiogenic properties of cartilage was performed in 1973 by Einsenstein and coworkers, who showed that cartilage extracted with 1 M guanidine lacked resistance to blood vessel invasion from the vascular network in the chick embryo chorioallantoic membrane (CAM) model.[10] Based on these findings, Brem and Folkman later found that a section of unprocessed cartilage tissue inhibited tumor-induced angiogenesis in the rabbit cornea when placed adjacent to the tumor.[11]

Using bovine and shark cartilage as sources, several components with antiangiogenic and antitumor activity have been obtained (Table 1). A partially purified fraction was isolated by Langer and coworkers from scapular cartilage of calf. This fraction contained a protein with a molecular weight of about 16 kDa, which inhibited tumor-induced angiogenesis in the rabbit cornea, and which was also shown to inhibit the growth of rabbit V2 carcinomas, as well as block blood vessel growth in the tumor.[12] Later, Lee and Langer realized that cartilage was present in only small quantities in mammalians, and therefore resorted to the use of Basking sharks (*Cethorinus maximus*) as a source of cartilage.[7] From this cartilage they obtained an extract that was incorporated into

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Table 1. Basic and Clinical Studies Evaluating the Antiangiogenic and Antitumor Activities of Cartilage-Derived Preparations

Author, year [REF.]	Preparation	Method	Activity
Brem, 1975 [11]	Crude cartilage	Rabbit Cornea Assay	Inhibition of tumor-induced angiogenesis
Lee, 1983 [7]	SC fraction	Rabbit Cornea Assay	Inhibition of tumor angiogenesis
Oikawa, 1990 [13]	SC fractions	Rabbit Cornea Assay, CAM	Inhibition of tumor angiogenesis
Moses, 1990 [14]	Calf Cartilage Protein (CDI)	EC culture, CAM	Inhibition of EC proliferation and migration
Mc Guire, 1996 [15]	SC extract	EC culture	Inhibition of EC proliferation
Davis, 1997 [25]	Powdered SC (oral)	Rat mesenteric window	Angiogenesis inhibition
Sheu, 1998 [16]	Purified fraction of SC (U-995)	CAM	Angiogenesis inhibition
		EC culture	Inhibition of EC proliferation and migration
		Collagenase Assay	Inhibition of collagenase activity
Dupont, 1998 [17]	Purified fraction of SC (AE-941, Neovastat)	Skin irritation model, CAM	Angiogenesis inhibition
		Collagenase Assay	Inhibition of collagenase activity
Miller, 1998 [29]	Powdered SC (oral)	Clinical trial (Cancer patients)	No significant response
Horsman, 1998 [35]	Powdered SC (oral)	SCCVII primary carcinoma in mouse	No significant response
Berbari, 1999 [26]	Liquid SC extract (oral)	Subcutaneous PVA implant in humans	Inhibition of EC proliferation
Gonzalez, 2001 [24]	Powdered SC (oral)	Rabbit Cornea Assay	Inhibition of bFGF-induced angiogenesis
	Water-soluble fraction		
		EC culture	Inhibition of EC proliferation

REF, reference; SC, shark cartilage; CAM, chorioallantoic membrane; EC, endothelial cell; PVA, polyvinyl alcohol; CDI, cartilage-derived inhibitor; bFGF, basic fibroblast growth factor.

slow-release pellets and tested for an inhibitory effect on neovascularization induced by rabbit V2 carcinoma and B16 melanoma in the rabbit cornea. The extract from Basking sharks strongly inhibited neovascularization induced by these tumors. Furthermore, the authors discovered that shark cartilage exerted those effects at a crude stage of purification in relation to calf cartilage. They calculated that shark cartilage contained about 100000 times more antiangiogenic activity compared to calf cartilage and recognized shark cartilage as a major natural source of angiogenesis inhibitors.[7)]

Additional evidence was afforded by Oikawa *et al*., who tested a cartilage extract obtained from the Japanese shark species *Glyphis glancus*. To obtain this cartilage-derived product they used a modification of the extraction method formerly published by Langer. The extract was tested for an inhibitory effect on tumor-induced neovascularization in rabbit cornea and in the CAM model. This crude extract was separated into four fractions, two of which showed antiangiogenic activity in the two models, with the major activity residing in fraction 3.[13)]

In the same year, Moses and coworkers reported the isolation of a protein with angiogenesis inhibitory activity from calf scapular cartilage using three different bioassays. This protein was partially sequenced and called cartilage-derived inhibitor (CDI). CDI inhibited endothelial cell proliferation and migration *in vitro* and blood vessel formation in the CAM model.[14)] Moreover, McGuire *et al*. found that an extract prepared from shark cartilage had antiproliferative activity specific for both ECGF-stimulated and quiescent human umbilical vein endothelial cells (HUVEC). This activity was observed only with fractions having a molecular weight less than 10 kDa.[15)]

More recently, two purified preparations have been obtained from shark cartilage, U-995 derived from the shark *Prionace glauca*,[16)] and AE-941[17)] (in the latter case the species of shark used was not indicated). Like other cartilage-derived preparations, U-995 showed a potent antiangiogenic activity in the CAM model, which seemed to be due to an inhibition of endothelial cell proliferation and migration and of collagenase activity. However, U-995 at a dose of 200 μg given orally 1—4 times per day did not affect the growth of sarcoma-180 solid tumors in mice. However, when administered intraperitoneally, U-995 inhibited the growth of these tumors in a dose-dependent manner. U-995 also showed a marked reduction of lung metastases of B-16–F10 melanoma when given i.p. at a dose of 200 μg per mouse. This effect was not observed with high doses of 400 μg or even 1 mg per mouse when the preparation was administered orally, suggesting that the active constituent is digested in the gastrointestinal tract.[16)]

AE-941 (Neovastat) seems to be the most promising preparation derived from shark cartilage. This compound presented antiangiogenic effects in the CAM model and was shown to have a potent inhibitory effect on collagenase activity. Further, Neovastat showed an efficient, preventive activity in a skin irritation model in humans, suggesting its potential utility in the treatment of psoriasis.[17)] Since 1998, a multicenter phase III clinical trial was approved by NCI in agreement with Aeterna Laboratories (Quebec, Canada) for evaluation of antiangiogenesis activity and antitumor efficacy of Neovastat in cancer patients.[18)] The final results of this clinical trial have not been published yet.

The results obtained from studies examining the mechanisms of action in the antiangiogenic and antitumor properties of cartilage-derived compounds have helped to reinforce the hypothesis that both endothelial cell activation leading to an increase in their proliferation and migration as well as an up-regulation of proteolytic enzymes such as plasmin and metalloproteinases are essential events for angiogenesis and tumor development (Fig. 1).[19,20)] An additional mechanism for the antiangiogenic properties of cartilage have been recently proposed by Moses and coworkers.[21)] These investigators found that the contractile protein troponin I (TnI) is present in human cartilage and proposed that TnI could inhibit the endothelial cell growth through the induction of changes in the shape of these cells and its affinity for heparin leading to competition with bFGF for this molecule on the endothelial cell surface.[21)] However, there is no report showing the presence of TnI in shark cartilage.

In our department a water-soluble fraction (WSF) was re-

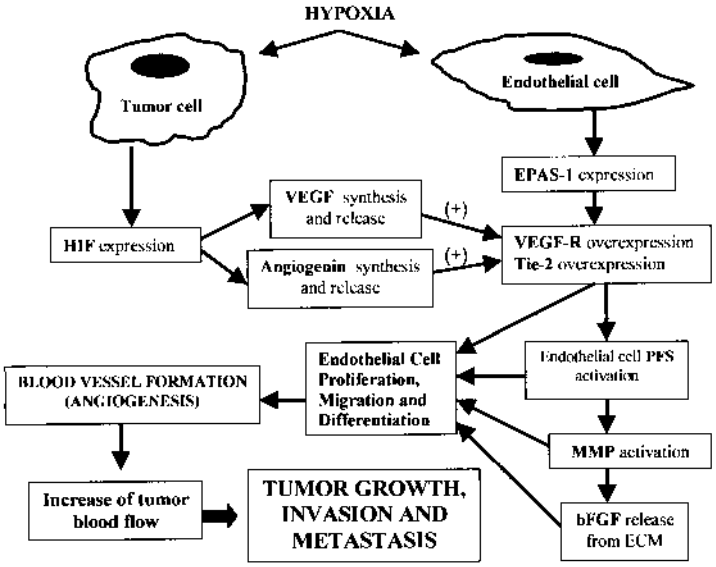

Fig. 1. Schematic Representation of the Role of Angiogenesis in Tumor Growth, Invasion and Metastasis

When the tumor grows beyond 2—3 cubic millimeters hypoxia causes the tumor cell to overexpress and release growth factors (VEGF, Angiogenin) that in turn stimulate the proliferation, migration and differentiation of endothelial cells leading to the formation of new blood vessels increasing tumor blood flow, which accelerates tumor growth and invasion of neighboring tissues and increases the probability of distant metastasis. HIF (hypoxia-inducible factor); EPAS-1 (endothelial Per-ARNT-Sim protein-1); VEGF (vascular endothelial growth factor); bFGF (basic fibroblast growth factor); VEGF-R (VEGF receptor); PFS (pericellular fibrinolytic system); MMP (matrix metalloproteinases); ECM (extracellular matrix); (+): stimulation.

cently obtained from shark cartilage. It was shown that WSF administered orally has anti-inflammatory and analgesic properties in classical animal models.[22] Later, the authors demonstrated that WSF was comprised of two main chromatographic peaks, one of which contained a peptide that could account for the pharmacological effects of WSF. Moreover, they showed that the L-arginine–nitric oxide pathway is involved in the mechanism of action of this fraction.[23]

In a recent study, we reported the antiangiogenic activity of this WSF in the rabbit cornea assay. WSF at 50 to 200 μg was incorporated into a slow-release pellet and placed inside of corneal pockets adjacent to the angiogenic stimulus. Using this model we found that WSF inhibited bFGF-induced angiogenesis in a time- and dose-dependent fashion. Also, we found that this antiangiogenic activity was due to the inhibition of endothelial cell proliferation. However, unlike other cartilage-derived products, WSF was not found to inhibit collagenase activity at concentrations of 100 and 200 μg/ml.[24]

The antiangiogenic activity of crude powdered shark cartilage (PSC), administered orally, has also been assessed in various animal models. Davis and coworkers used a modification of the rat mesenteric window assay as an angiogenesis model to study the effects of two commercial crude shark cartilage products obtained from blue shark. Rats were given orally 600 mg/kg PSC for two weeks prior to the induction of angiogenesis. This led to the inhibition of angiogenesis induced in the nearly avascular mesenteric windows by the mast cell secretagogue compound 48/80. They also demonstrated a direct relationship between the dose ingested by the animals and the rate of angiogenesis inhibition, suggesting that the active antiangiogenic factor of shark cartilage was being absorbed at the level of the intestinal wall in a non-saturated manner at the doses studied.[25] However, it was not certain whether the active compound was a substance that was absorbed intact or whether it was a product of metabolic transformation before or during its absorption.

Additional evidence of gastrointestinal absorption after oral administration of a liquid cartilage extract (LCE) derived from shark, was recently demonstrated in humans. LCE was able to reduce the endothelial cell density inside a polyvinyl alcohol (PVA) implant inserted subcutaneously in the left arm of healthy volunteers.[26] Although these authors did not measure blood vessel formation directly, the inhibitory action of LCE on endothelial cell density was regarded as assumed as an antiangiogenic effect.

We have also tested the antiangiogenic effect of a commercial powdered shark cartilage (PSC) (Selachii Produtos Marinhos Ind. ltda, Fortaleza), given orally, in a modification of the rabbit cornea assay in which basic fibroblast growth factor was used as the angiogenic stimulus. This PSC was obtained from the same species of shark used to obtain the WSF. In this work PSC was given by gavage at a dose of 100 mg/kg/d during 27 d (12 d before and 15 d after the implantation of bFGF-bearing pellets). Our results showed a strong antiangiogenic effect of PSC that produced as much as 95% inhibition, affording more evidence to support the feasibility of gastrointestinal absorption of antiangiogenic factor(s) in shark cartilage.[24]

Taken together all these results strongly suggest that shark cartilage is a source of several compounds with potent antiangiogenic activity and that some of these active principles can be absorbed through the intestinal wall when given orally. This hypothesis is also supported by the findings showing that macromolecules such as proteins can cross the gastrointestinal barrier in amounts that remain biologically active and immunogenic. It has been shown that albumin and β-lactoglobulin are transported through luminal or intercellular routes in small but significant quantities and that the rate of absorption is related to the amount of protein ingested and to some macromolecular characteristics such as molecular weight.[27,28]

Conflicting Results in Studies with Shark Cartilage

Although there is an increasing body of evidence showing that shark cartilage has potent antiangiogenic and antitumoral activities, some negativity can be found in the scientific literature concerning the usefulness of shark cartilage and derived compounds in the treatment of cancer and/or other angiogenesis-dependent diseases. This contrary viewpoint comes principally from two aspects: first, the lack of positive convincing results in clinical trials with the use of shark cartilage derived products, and second, the notion that cartilage-derived polypeptide factors are insufficiently absorbed through the intestinal wall after oral administration.

A few clinical studies have evaluated the efficacy and safety of shark cartilage in the treatment of cancer patients.[18,29,31] However, some of these studies have been severely criticized because they did not follow randomized and placebo-controlled protocols.[30] Also, in some cases, the final sample size was very small with no significant results. Furthermore, except for the trial conducted by Miller *et al.*,[29] the results obtained in these clinical studies have not been published in peer-reviewed journals. These facts have led to inconclusive and unreliable results.

In particular, the phase I/II trial carried out by Miller *et al*., evaluating the safety and efficacy of shark cartilage (1 g/kg) orally administered to advanced cancer patients, showed no beneficial effects in the quality of life of those patients using shark cartilage as a single therapeutic agent.[29]

In spite of these discouraging results, the use of complementary medications, including shark cartilage, have increased among cancer patients as shown in a survey performed among 143 advanced cancer patients in which the authors found that shark cartilage was used by 21 (10.7%) of those patients.[32] However, there has outspoken criticism of the shark cartilage use in cancer patients in light of the lack of sufficient clinical evidence. For example, Ernst from the Department of Complementary Medicine of the University of Exerter, U.K., has manifested his opposing opinion to the use of shark cartilage in cancer treatment, in a letter sent to the editors of The Lancet in 1998, whereby he emphasized the lack of reliable dose-response results and of bioavailability studies supporting the usefulness of this product. Also, he questioned a clinical trial with cancer patients that, according to him, began in 1994 and was conducted by the NCI (U.S.A.) using "100 mg" daily powdered shark cartilage, arguing that the results were not published in any peer-reviewed journal.[33] However, that allegation was quickly answered and denied by the Simone Protective Cancer Institute because the trial was not performed.[34]

Conflicting results in pre-clinical studies with cartilage-derived products have also been obtained. In a study performed by Horsman *et al*., two different extracts from commercially available shark cartilage (Sharkilage and MIA Shark Powder) were tested for the antitumor and antimetastasic effects. The products were administered orally to mice at doses from 5 to 100 mg/daily per mouse for 25 d after the implantation of SCCVII primary carcinoma. They found no decrease in either the growth of the primary tumor or inhibition of lung metastasis in the mice.[35]

The second conflicting point is related to the administration route of shark cartilage and derived compounds. Several investigators have argued against the efficacy of shark cartilage administered orally. The argument comes from the fact that some of the components found in shark cartilage having antiangiogenic and antitumor activity are peptides or proteins. Thus, these compounds would not be absorbed through the intestinal wall due to proteolytic degradation.[33] However, a better understanding about the digestion process has afforded evidence supporting the notion that this proteolytic process is not totally efficient. As noted above, intact macromolecules, even insulin, can cross the intestinal barrier in a biologically active form and thus exert their pharmacological actions.[27,28] This absorption process may also occur with the active compounds of shark cartilage.

In spite of the absence of bioavailability studies, the intestinal absorption of cartilage-derived compounds has been strongly supported by several recent experimental findings of the pharmacological activity of powdered shark cartilage or derived compounds administered orally.[22—26]

CONCLUSIONS

In this review we have shown that several *in vivo* and *in vitro* experimental studies have demonstrated that shark cartilage is a true source of biological compounds with antiangiogenic and antitumor properties. Unfortunately, those experimental findings have not been followed by reliable results in clinical trials, especially with cancer patients. The later contradiction could be the cause of the skepticism in relation to the therapeutic properties of shark cartilage and derived compounds. One objective of the present paper is to analyze the possible explanations for these contradictory results by addressing one logical question: If shark cartilage derivatives have been shown to be effective both in *in vitro* and *in vivo* models as antiangiogenic and antitumor drugs, why have they not been proven clinically effective in the treatment of human disease? In this sense, several important issues must be examined. First, angiogenesis is a redundant process meaning that it can be triggered by several angiogenic factors functioning through different and complex signal pathways which are not totally understood at present. Thus, the antiangiogenic activity of a single agent can be limited by metabolic pathways participating in the angiogenic process but not affected by the action of the current therapeutic agent. In fact, it has been proposed that combinations of antiangiogenic compounds with traditional antineoplasic drugs could lead to better results in the treatment of cancer than with the use of each product alone.[36]

The second critical issue to be considered is the mode of administration of the compound, because some of the antiangiogenic compounds found in cartilage seem to be proteins that could be partially or totally destroyed in the gastrointestinal tract when administered orally. Furthermore, differences in the mechanisms of gastrointestinal absorption and metabolism between the experimental animals and humans may account for the contradictory results obtained.

Third, although Folkman's hypothesis that tumor growth depends entirely on new capillary formation is well established, there is evidence indicating that this view is not totally true. It was shown that in some types of tumors such as in non-small cell lung cancer, tumor growth follows four different patterns of vascularization. For instance, in 16% of these tumors the tumor cells grow along the pre-existing vascular bed without indication of active angiogenesis.[37] Also,

other tumors are able to develop two growth patterns simultaneously, namely angiogenesis-dependent and angiogenesis-independent.[38] These facts can be combined to make this type of tumor resistant to antiangiogenesis therapy.

Fourth, clinical studies reported in the literature have been performed in patients with very advanced cancers, many of them in terminal phases, which can have a negative impact on the outcome of any treatment.

To eventually resolve the question of whether shark cartilage offers a potential effective cancer treatment, two issues must be addressed. First, it will be helpful to determine the molecular structure of these cartilage-derived anti-angiogenic compounds and synthesize new drugs that can be administered systemically to improve bioavailability. Secondly, it will be necessary to submit antiangiogenic drug candidates to standard clinical trials, rigorously controlled, randomized and double-blinded to obtain reliable results in the future.

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