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Shark cartilage extracts as antiangiogenic agents: smart drinks or bitter pills?

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Abstract

The use of crude cartilage for the treatment of human cancers remains a subject of controversy. In this brief commentary, we reviewed the current knowledge on the anticancer properties of cartilage. We then presented the properties of Æ-941, a novel standardized water-soluble extract derived from shark cartilage that represents less than 5% of the crude cartilage. It is a multifunctional antiangiogenic product that contains several biologically active molecules. Æ-941 is one of the few antiangiogenic drugs that is under phase III clinical investigation. It is currently evaluated in Europe and North America for the treatment of refractory renal cell carcinoma and in North America for metastatic non small cell lung cancer.

Abbreviations: MMP – matrix metalloproteinase; TIMP – tissue inhibitor of metalloproteinase; VEGF – vascular endothelial growth factor.

Controversy and scientific reality

There is still considerable controversy regarding the clinical usefulness of cartilage for the treatment of cancer. Most of this controversy arises from the false claims that sharks (elasmobranch family) which skeleton is made by cartilage (about 6% of the whole body weight) do not develop cancers and that solid forms of shark cartilage may cure cancer in animals and humans. As a result, a large variety of rather expensive powders from total preparations of cartilage have invaded the natural product market. Over the last 25 years, a number of studies have claimed that powdered cartilage extracts were efficient for the treatment of cancer patients but most of these studies were received with specticism by the scientific community due to their lack of adequate protocols. In the rare cases where these studies were conducted with a significant number of patients, no consistent improvement of patient conditions could be observed [1]. A recent Phases I/II clinical trial on cancer patients failed to support a beneficial effect of shark cartilage powder [2]. Moreover, the precise composition of these crude preparations are unknown and the biological activities supporting the use of such products in the cure of solid tumors and other related diseases have never been established.

On the other hand, cartilage from all species is an avascular tissue known to develop malignant tumors only in rare occasions [3–6]. This resistance of cartilage to tumor formation seems to be correlated to the capacity of the tissue to inhibit the formation of new blood vessels which are necessary for the growth and the metastatic power of tumor cells [7–9]. In a series of experiments, Brem and Folkman [10] demonstrated that fractions or liquid extracts of cartilage were able to inhibit the proliferation of capillaries induced by tumor cells. Subsequently, it was shown that a cartilage fraction extracted by guanidium and purified by affinity chromatography was also able to inhibit angiogenesis induced by tumor cells [11,12]. This property of cartilage is conserved throughout evolution as antiangiogenic activities were observed in several species such as shark [13–16], bovine [10], and human [17]. Although the mechanisms involved in these antitumor

properties of cartilage extracts are not understood in detail, the purification of some of cartilage antitumor factors have suggested the potential involvement of anti-collagenase activities in their effects [16,18–22]. The antiangiogenic properties of bovine cartilage extracts were mainly correlated with the presence of at least two distinct type I collagenase inhibitors, named CDI and BCDI [18,23], that share extensive homologies with TIMP-1 and TIMP-2, respectively. This has led to the suggestion that these MMP inhibitors may account for many of the antiangiogenic properties of cartilage and may thus represent attractive therapeutic anticancer agents [19]. In the last decade, other cartilage-associated proteins with antiangiogenic properties such as chondromodulin [24], troponin I [25] and thrombospondin I [26] have been identified, further emphasizing the potential use of cartilage as an abundant source of inhibitors of neovascularization and therefore of potential anticancer drugs. However, the therapeutic potential of cartilage extracts relies on the adequate extraction of these molecules as well as on the preservation of their biological activities following their extraction and storage. In this respect, it is likely that commercial shark cartilage powders that contain a high proportion of inorganic material may have a low content in these inhibitors that would explain their low efficacy.

Æ-941, a novel water-soluble shark cartilage extract with antiangiogenic properties

Æ-941 is a standardized shark cartilage extract produced and tested according to CGMP criteria. Æ-941 differs from the previously reported solid extracts since it is a water-soluble extract from which more than 95% of inactive molecules have been removed, resulting in high concentrations of biologically-relevant molecules [27]. Following extraction, soluble molecules are separated from the solid part by centrifugation, and ultrafiltration is performed with a cut-off of 500 kDa to obtain the liquid extract, the Æ-941, and the extract is kept frozen until use to maximally preserve its biological properties. This mode of extraction thus differs considerably from that of other commercial preparations and may explain the preservation of the antiangiogenic properties of Æ-941. In particular, it was demonstrated that the compound has a marked inhibitory effect on embryonic vascularization (Figure 1), endothelial cell proliferation [28,29] and tubulogenesis [30]. The antiangiogenic activity of Æ-941 was correlated with the presence of an antimetalloproteinase inhibitor that inhibits several members of the MMP family, with a preferential inhibition of MMP-2 (Figure 2A) as well as with that of a serine elastase inhibitory activity (Figure 2B). In addition,

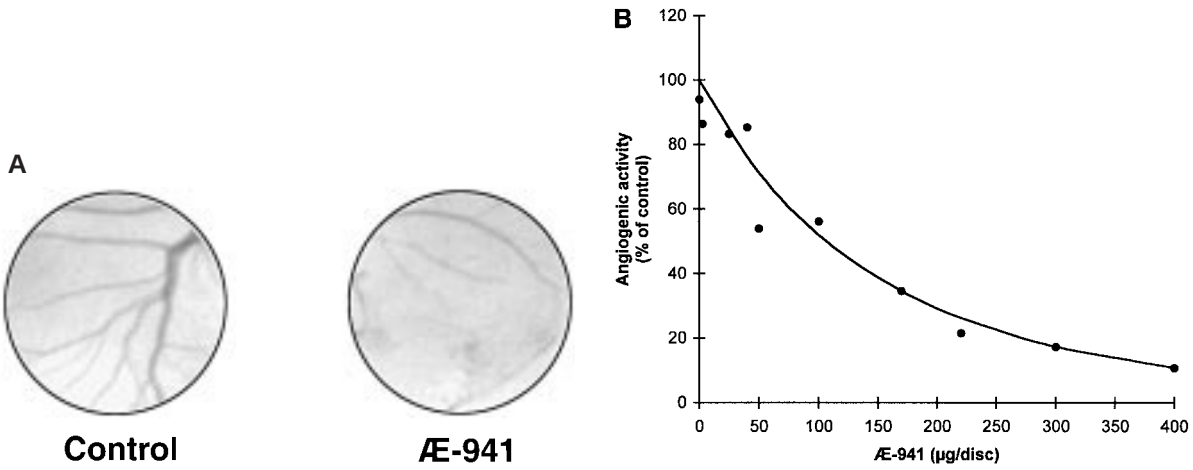

Figure 1. Effect of Æ-941 on embryonic neovascularization. (A) *Ex ovo* chick embryo culture were treated with methylcellulose disk containing either a control solution or 200 µg Æ-941. Discs were deposited on the external border of the vascular perimeter near the tip of blood vessels, where the angiogenic process is active. In order to minimize inter-individual variations, discs containing either the drug sample or the vehicle were placed in a symmetric fashion with respect to the cephalo–caudal axis of the embryo, since blood vessels develop symmetrically to that axis. *Ex ovo* incubation of the embryos was pursued for 24 h. Ten chick embryos per conditions were examined. (B) Increasing concentration of Æ-941 were added to the chick embryo and angiogenesis was assessed in a blind semi-quantitative fashion. The blood vessel formation was considered as altered when its growing path was either deviated, diminished or when there was no growth observed beyond the disc as compared to the negative control.

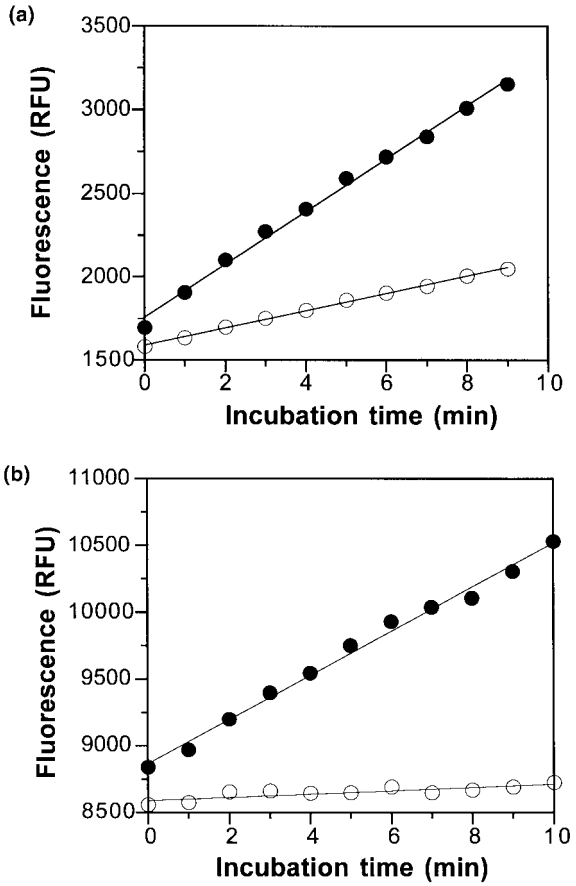

Figure 2. Effect of Æ-941 on the gelatinolytic and elastinolytic activities of matrix metalloproteinase-2 and serine elastases. Recombinant MMP-2 (A) or porcine pancreatic elastase (PPE) (B) were incubated in the absence (dark circles) or in the presence (open circles) of 100 μg/ml of Æ-94l in a reaction buffer containing gelatin or elastin labeled with BODIPY FL. The proteolytic activities of both enzymes were determined by following the increase in fluorescence at 538 nm. Under these conditions, Æ-941 inhibited MMP-2 and PPE activities by 60% and 98%, respectively.

Æ-941 inhibits VEGF binding to endothelial cells [31], the VEGF-dependent tyrosine phosphorylation of the VEGF receptor as well as the VEGF-dependent increase in vascular permeability [30].

In vivo, Æ-941 was also found to have significant *in vivo* antitumor and antimetastatic activities [32]. In a therapeutic point of view, recent results from a phases I–II clinical trial in patients suffering from a refractory non-small cell lung cancer revealed that Æ-941 is orally bioavailable and safe, since no serious side effects related to the administration of Æ-941 have been observed. Furthermore, a retrospective analysis indicates that Æ-941 significantly increase the median survival of these patients [33]. Æ-941 is one of the few antiangiogenic drugs that has reached phase III clinical trial evaluation. It is currently tested in phase III clinical trials in Europe and North America for the treatment of refractory renal cell carcinoma and in North America for metastatic lung cancers.

Conclusions

Scientific data obtained from various studies where Æ-941 has been tested strongly suggest that controlled water extraction and frozen storage of cartilage represent crucial steps in order to retain its multiple biological activities. By contrast to powdered shark cartilage preparations that show no significant effects on cancer patient's conditions, Æ-941 has been used in well-controlled clinical settings and emerged as a compound that can significantly improve survival. Given the recent studies supporting the presence within Æ-941 of specific inhibitor(s) of VEGF-mediated signal transduction events together with proteinases inhibitors, this compound appears as a multitarget drug contrasting from other natural or chemical antiangiogenic agents by its efficiency against several crucial steps of the angiogenic cascade closely linked to tumor progression. Further characterization and purification of the molecular components that are responsible of the biological activity of Æ-941, including the inhibition of specific matrix-degrading enzymes, serine elastases, and the control of endothelial cell proliferation will be of significant importance in our understanding of the molecular and cellular mechanisms involved in the antiangiogenic and antimetastatic properties of Æ-941.

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