The Effect of Shark Cartilage Extracts on the Growth and Metastatic Spread of the SCCVII Carcinoma

Michael R. Horsman, Jan Alsner and Jens Overgaard

From the Danish Cancer Society, Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark

Correspondence to: Michael R. Horsman, Ph.D., D.M.Sc., Danish Cancer Society, Department of Experimental Clinical Oncology, Aarhus University Hospital, Nørrebrogade 44, Bldg. 5, DK-8000 Aarhus C, Denmark. Tel: + 45 89 49 26 22. Fax: + 45 86 19 71 09.

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This study was designed to investigate the potential of shark cartilage extracts to inhibit the growth and metastatic spread of a murine solid tumour. The SCCVII carcinoma, implanted in the right rear foot of C3H mice, was used. Following tumour implantation, two different commercially available extracts of shark cartilage (Sharkilage and MIA Shark Powder) were dissolved in water and orally administered to the mice at doses that ranged from 5 to 100 mg per mouse. These injections were repeated on a daily basis for up to 25 days post-implantation of the primary tumour. Compared to non-drug-treated animals, daily administration of the shark cartilage extracts did not show any adverse toxicity (as measured by changes in body weight and lethality). More importantly, none of the shark cartilage doses tested had any retarding effect on the growth of the primary tumour, nor did they inhibit the development of metastases seen in the lungs of the tumour-bearing mice at autopsy. In conclusion, our results offer no support for the proposed use of shark cartilage extracts as an anti-cancer therapy.

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There has been a great deal of media attention recently focused on the use of shark cartilage as a potentially new anti-cancer treatment. The issue is a very sensitive one. On the one side, there are those that claim that there is evidence from both animal and human studies demonstrating the anti-tumour activity of shark cartilage, and on the other, those that claim that no such clear evidence exists and that the mass slaughter of sharks is not justified (1–4). However, the concept that shark cartilage may have the potential to inhibit the growth of solid tumours does have some scientific foundation.

There is good evidence that the growth of primary tumours, and the spread and subsequent growth of their metastases, is dependent on angiogenesis (5–7). This is a highly complex process by which tumours develop their own blood supply from the normal tissue blood vessels (5, 8, 9). Considerable effort has been made to find agents that could specifically inhibit this angiogenic process (9, 10). One substance that appears able to do this is cartilage. Initial studies demonstrated that rabbit neonatal scapular cartilage could inhibit the neovascularization of tumours implanted into the cornea of rabbits (11). Additional studies were able to isolate a factor from bovine cartilage that could inhibit the neovascularization in the rabbit corneal assay (12, 13), in the subconjunctival of mice (13) and in the CAM assay (14). Later it was also shown that shark cartilage contained inhibitors that could inhibit angiogenesis measured with the rabbit corneal assay (15) and a human umbilical vein endothelial cell proliferation assay (16).

What has never been shown in any peer reviewed publication is the ability of shark cartilage per se to inhibit tumour growth and development in vivo. The current study was a preliminary investigation in mice to test the anti-tumour activity of two commercially available shark cartilage extracts. Treatments involved daily oral administration of these agents, and were designed to duplicate as closely as possible the recommended human application. Tumour response was observed using the SCCVII murine carcinoma model. This particular tumour model was selected because it can be grown as a solid tumour known to be responsive to both conventional anti-cancer treatments (17–21) and anti-angiogenic therapy (22). Furthermore, this tumour readily gives rise to pulmonary metastases, the development of which can be modified by therapeutic intervention (23, 24).

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MATERIAL AND METHODS

Animals and tumours

Female C3H/bom mice, aged between 3 and 4 months and weighing approximately 27 g, were used in all experiments. The tumour used was the SCCVII carcinoma, the derivation of which has been previously described (17). This tumour was maintained by serial transplantation on the flanks of C3H mice, fresh tumours being taken from frozen stock every 3 months. Experimental tumours were produced following sterile dissection of the large flank tumours. Macroscopically viable tumour tissue was minced with a pair of scissors, and 5–10 μl of this material injected subcutaneously into the foot of the right hind limb of the experimental animals. The tumour take in this study was 100%. All the experiments were performed under nationally approved guidelines for animal welfare.

Shark cartilage extracts

Two commercially available extracts of shark cartilage were used. These were Sharkilage (Futurebiotics, Vermont, USA) and MIA Shark Powder (MIA Export Ltd., Australia). Both types were dissolved in sterile water at a range of concentrations and orally injected into mice at a volume of 0.5 ml/mouse, on a daily basis. Control mice were given either no treatment or daily injections of sterile water.

Treatment and response

The body weights of all animals were recorded prior to the start of the experiment. Mice were then implanted with the SCCVII tumours and orally injected with the appropriate dose of the shark cartilage extract. These injections were repeated on a daily basis for up to 25 days post-implantation. During this time both the mouse body weights and tumour volume were recorded daily. Tumour volume was determined by the formula $D_1 \times D_2 \times D_3 \times \pi/6$ (where the D values represent 3 orthogonal diameters). At the end of this 25-day period, the mice were killed by cervical dislocation. This was necessary at this time to prevent the primary tumour becoming too large or the metastatic burden too severe. The lungs of the killed mice, and those mice dying before the end of the 25-day period, were removed and examined for metastases. Scoring of these metastases was done on an arbitrary scale of no, few or many obvious metastases.

RESULTS

The growth of control SCCVII tumours following foot implantation is shown in Fig. 1. Initially there was a lag phase of approximately 1 week, after which the tumours grew rapidly until they reached a size, typically around 600 mm^3, when tumour growth appeared to slow. By day 25, the average tumour volume was 1000 mm^3. During the first 10-day period of tumour growth, mouse body weight shows a slight, but non-significant (Student's t-test; $p > 0.05$) decrease from the average starting value of 27.0 down to 26.4 g. However, after 25 days there is a highly significant ($p < 0.001$) drop to 22.8 g.

Fig. 2 illustrates the effect that daily administration of shark cartilage had on mouse body weight. The 15–20% decrease in mouse body weight seen in the control animals during the 25 days post-tumour implantation period was not significantly changed ($p > 0.05$) by daily administration of any dose of either shark cartilage extract. A total of 28% of control animals died during the 25-day treatment period, and again there was no obvious effect of treating with the different shark cartilage extracts on this rate of lethality (data not shown).

The ability of the different shark cartilage extract treatments to inhibit the growth of the foot implanted SCCVII tumour was also investigated. Tumour volume was measured daily after implantation, as shown in Fig. 1, and the time taken to reach a volume of 500 mm^3 was recorded. On average, it took 14 days for control tumours to reach this volume. The effect of the different shark cartilage extract treatments on the time taken to reach this size is shown in Fig. 3. Any inhibition of tumour growth would be evidenced by values significantly greater than 1.0. None of the shark cartilage extract doses used in this study had

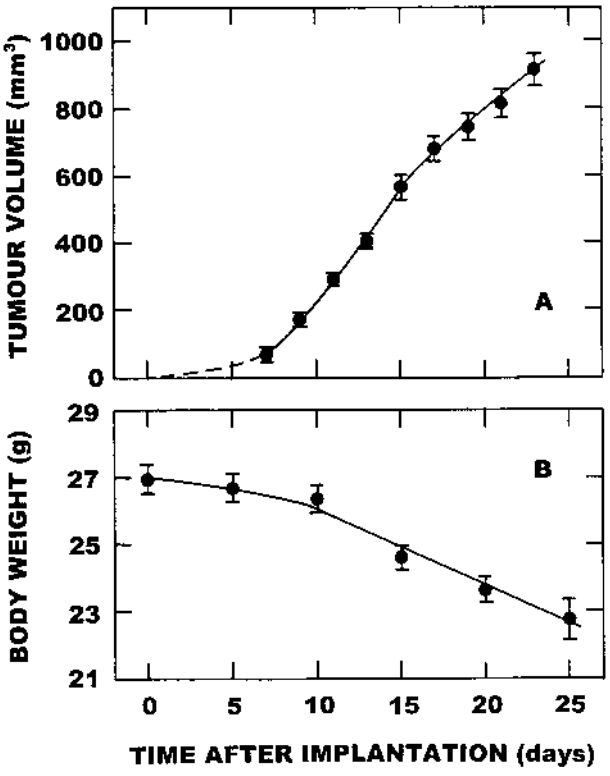

Fig. 1. Tumour volume (A) and mouse body weight (B) measured in control mice as a function of time after implantation of SCCVII tumour material in the foot of the host animals. Results include both non-treated controls and those control mice that received daily oral injections of saline. Points are means ($\pm$1 SE) calculated from an average of 32 mice/group.

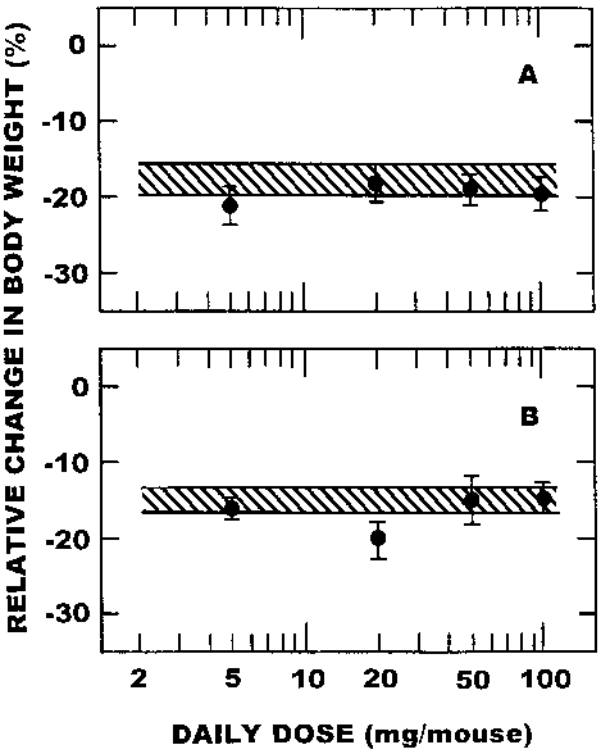

Fig. 2. The effect of shark cartilage extracts on mouse body weight. Results show the relative percent of change in body weight, between the weight measured prior to drug injection and that measured on the last day of injection with different doses of either Sharkilage (A) or MIA Shark Powder (B). Points are means (± 1 SE) calculated from an average of 12 mice/group. The shaded area shows the SE (± 1) for control animals.

any significant ($p > 0.05$) inhibitory effect on the growth of the primary SCCVII tumour.

Table 1 shows the potential of these shark cartilage extract treatments to influence metastatic spread of this SCCVII tumour. In the control tumours, there was a difference between the two series of experiments. For the Sharkilage study, the percentage of animals showing no obvious metastases was 32%, but in the MIA Shark Powder study, this percentage was higher at 70%. What is clear is that none of the shark cartilage extract treatments had any influence on metastatic spread when compared to controls in the same series of experiments. There was no increase in the number of animals showing no obvious metastases, nor was there any reduction in the number of animals in which the lungs showed many metastases.

DISCUSSION

In this study we found that daily oral administration of two different commercially available brands of shark cartilage extracts, at doses of between 5 and 100 mg/mouse, were well tolerated by the animals. More significantly, these doses had absolutely no detectable effect on the growth of the transplanted SCCVII murine carcinoma, nor did they influence the metastatic spread of this tumour to the lungs of the host animals. The SCCVII tumour model was selected for this study because it has been well documented, using both in vitro/in vivo excision and tumour growth delay assays, that primary tumours are responsive to conventional anti-cancer therapies such as radiation (19, 20), chemotherapeutic drugs (17, 18) and hyperthermia (21, 22). Growth of this SCCVII tumour has also been shown to be reduced by the anti-angiogenic fumagillin analogue, TNP-470 (22). Finally, this tumour is known to give rise readily to metastases in the lungs of the host animals, and the development of these pulmonary metastases can also be reduced by various treatments (23, 24).

Only one other experimental study has investigated the effect of giving shark cartilage to mice: the one referred to in the book by Lane & Comac (3). In that study, the growth of human melanoma xenografts was inhibited with daily oral administration of 1200 mg/kg shark cartilage. Our study found no effect with oral daily doses of 5–100 mg/mouse, which, for a 27-g mouse, would be equivalent to 185–3704 mg/kg. The reason for the difference in results between the two studies is not clear, but it is

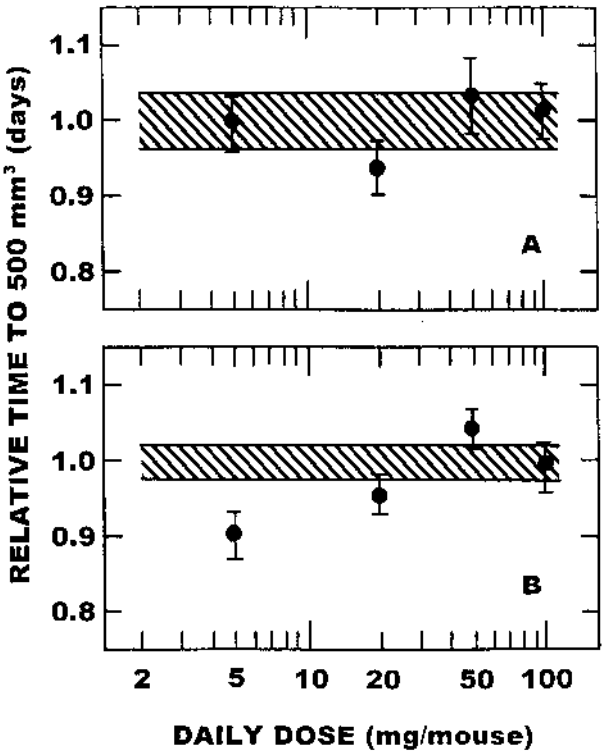

Fig. 3. The effect of different shark cartilage extract doses on the growth of foot implanted SCCVII carcinomas. Results show the time taken for tumours to reach a volume of 500 mm^3 following daily oral administration of either Sharkilage (A) or MIA Shark Powder (B), and are expressed relative to the time taken for control tumours to achieve this volume. Points are means (± 1 SE) calculated from an average of 11 mice/group. The shaded area shows the SE (± 1) for control animals.

Table 1

The effect of daily oral administration of different doses of shark cartilage extracts on the development of SCCVII metastases in the lungs of C3H mice

Type of shark cartilage extract[a]	Dose given (mg/mouse)[b]	SCCVII metastases[c]		
		No obvious (%)	Few (%)	Many (%)
A	0	6/19 (32)	8/19 (42)	5/19 (26)
A	5	2/9 (22)	5/9 (56)	2/9 (22)
A	20	2/9 (22)	5/9 (56)	2/9 (22)
A	50	3/10 (30)	6/10 (60)	1/10 (10)
A	100	1/10 (10)	7/10 (70)	2/10 (20)
B	0	14/20 (70)	5/20 (25)	1/20 (5)
B	5	5/9 (56)	3/9 (33)	1/9 (11)
B	20	5/10 (50)	2/10 (20)	3/10 (30)
B	50	7/10 (70)	2/10 (20)	1/10 (10)
B	100	8/10 (80)	2/10 (20)	0/10 (0)

[a] Mice were given either Sharkilage (A) or MIA Shark Powder (B).
[b] The shark cartilage extracts were administered orally at these doses on a daily basis up to 25 days after implantation of the primary tumour in the right rear foot.
[c] Results show the number of animals (and percentage) developing metastases in the lungs of SCCVII tumour bearing mice at the end of treatment.

interesting to speculate as to why such a seemingly positive finding, from a study that was completed in 1989, has never been published in any peer reviewed journal.

In patients, the usual oral daily dose seems to be around 1000 mg/kg (3, 25). It is difficult to know how to compare the human doses directly with those used in mice. On a purely weight basis, 1000 mg/kg in mice would be ineffective. However, if the conversion of the daily human dose to a mouse equivalent is based on surface area, then the equivalent mouse concentration would be higher by a factor of 12 (26), thus making 1000 mg/kg in man the same as 12000 mg/kg in mice. This is just over three times higher than the maximum concentration used in our experiments. In our study, higher doses were not possible due to solubility limitations of the shark cartilage, and because 0.5 ml was the maximum volume that could be orally administered to the mice. It is not clear whether the conversion of the human dose data to a mouse equivalent dose should be done on a weight or surface area basis. This can only be determined by performing detailed pharmacokinetic analysis to determine the relative concentration of the active component in the blood of mice and humans. However, since it is not clear exactly what active component one should look for, such an investigation is not possible at this time.

The early studies that demonstrated that shark cartilage could inhibit angiogenesis did not use cartilage per se, but rather substances that were isolated from it (15, 16). Moreover, these extracts were incorporated into pellets that were implanted into the cornea immediately in front of the tumours whose growth they inhibited. It is likely that, following oral administration of shark cartilage, the quantity of active substance that actually gets into the bloodstream and eventually reaches the tumour is probably much less than those used in the corneal experiments, and may be insufficient to have any inhibitory effect on the angiogenic process. This would argue against the oral ingestion of high doses of shark cartilage, and instead would support attempts to identify the active factors, reproduce them chemically and administer them directly to the tumour-bearing subjects. However, this would be no different from the studies already ongoing with angiogenic inhibitors such as angiostatin (27, 28), thrombospondin (29) and endostatin (30, 31).

In conclusion, our study could not detect any effect of oral administration of shark cartilage extracts on the growth and metastatic spread of at least one murine tumour model. Taking these data into consideration, along with the facts that there has never been any other published experimental data demonstrating the benefit of shark cartilage per se as an anti-tumour agent; that although experimental studies in the 1980s indicated a rationale for shark cartilage extracts having the potential to act as anti-angiogenic agents, there has never been any real follow-up; and that the only controlled clinical study investigating this aspect failed to show any benefit (25), it leads us to conclude that the use of shark cartilage extracts in cancer therapy has no documented effect.

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REFERENCES

1. Mathews J. Media feeds frenzy over shark cartilage as cancer treatment. J Natl Cancer Inst 1993; 85: 1190–1.
2. Mathews J. Sharks still intrigue cancer researchers. J Natl Cancer Inst 1992; 84: 1000–2.
3. Lane IW, Comac L. Sharks don't get cancer. New York: Avery, 1993.
4. Markman M. Shark cartilage: the Laetrile of the 1990s. Cleve Clin J Med 1996; 63: 179–80.
5. Blood CH, Zetter BR. Tumor interactions with the vasculature: angiogenesis and tumor metastasis. Biochim Biophys Acta 1990; 1032: 89–118.
6. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990; 82: 4–6.
7. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Med 1995; 1: 27–31.
8. Folkman J. The vascularization of tumors. Sci Am 1976; 234: 58–73.
9. Paweletz N, Knierim M. Tumor-related angiogenesis. Crit Rev Oncol Hematol 1989; 9: 197–242.
10. Mahadevan V, Hart IR. Metastasis and angiogenesis. Rev Oncol 1990; 3: 97–103.
11. Brem H, Folkman J. Inhibition of tumor angiogenesis mediated by cartilage. J Exp Med 1975; 141: 427–39.
12. Langer R, Brem H, Falterman K, Klein M, Folkman J. Isolation of a cartilage factor that inhibits tumor neovascularization. Science 1976; 193: 70–2.
13. Langer R, Conn H, Vacanti J, Haudenschild C, Folkman J. Control of tumor growth in animals by infusion of an angiogenesis inhibitor. Proc Natl Acad Sci USA 1980; 77: 4331–5.
14. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization from cartilage. Science 1990; 248: 1408–10.
15. Lee A, Langer R. Shark cartilage contains inhibitors of tumor angiogenesis. Science 1983; 221: 1185–7.
16. McGuire TR, Kazakoff PW, Hoie EB, Feinhold MA. Antiproliferative activity of shark cartilage with and without tumor necrosis factor-alpha in human umbilical vein endothelium. Pharmacotherapy 1996; 16: 237–44.
17. Hirst DG, Brown JM, Hazelhurst JL. Enhancement of CCNU cytotoxicity by misonidazole: possible therapeutic gain. Br J Cancer 1982; 46: 109–16.
18. Hirst DG, Horsman MR, Brown JM, Hazelhurst J. The effect of timing on chemosensitization by clinical levels of SR-2508. Int J Radiat Oncol Biol Phys 1984; 10: 1641–5.
19. Horsman MR, Wood PJ, Chaplin DJ, Brown JM, Overgaard J. The potentiation of radiation damage by nicotinamide in the SCCVII tumour in vivo. Radiother Oncol 1990; 18: 49–57.
20. Grau C, Nordsmark M, Khalil AA, Horsman MR, Overgaard J. Effect of carbon monoxide breathing on hypoxia and radiation response in the SCCVII tumor in vivo. Int J Radiat Oncol Biol Phys 1994; 29: 449–54.
21. Wood PJ, Horsman MR, Khalil AA, et al. A comparison of the physiological effects of RSU 1069 and RB 6145 in the SCCVII murine tumour. Acta Oncol 1996; 35: 989–94.
22. Nishimura Y, Murata R, Hiraoka M. Combined effects of an angiogenesis inhibitor (TNP-470) and hyperthermia. Br J Cancer 1996; 73: 270–4.
23. Ramsey J, Suit HD, Sedlacek R. Experimental studies on the incidence of metastases after failure of radiation treatment and the effect of salvage surgery. Int J Radiat Oncol Biol Phys 1988; 14: 1165–8.
24. Ogawa Y, Goodman GB, Chaplin DJ, Grulkey W, Lam GK. Combination therapy of pions and SPG (Sonifilan, Schizophyllan), a biological response modifier for mouse tumor systems. Int J Radiat Oncol Biol Phys 1990; 18: 1415–20.
25. Miller DR, Granick JL, Stark JJ, Anderson GT. Phase I/II trial of the safety and efficacy of shark cartilage in the treatment of advanced cancers. ASCO Proceedings 1997; 16: 49a.
26. DeVita VT. Principles of chemotherapy. In: DeVita VT, Hellman S, Rosenberg SA, eds. Cancer: principles and practice of oncology, 3rd ed. Philadelphia: JB Lippincott, 1989: 276–300.
27. O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a lewis lung carcinoma. Cell 1994; 79: 315–28.
28. Gately S, Twardowski P, Stack MS, et al. Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor, angiostatin. Cancer Res 1996; 56: 4887–990.
29. Good DL, Polverini PJ, Rastinejad F, et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc Natl Acad Sci USA 1990; 87: 6624–8.
30. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997; 88: 277–85.
31. Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 1997; 390: 404–7.