Effects of Oral Consumption of Shark Cartilage on the Development of Melanomas in Mice

Douglas Hopek

Memorial Sloan Kettering Hospital

Abstract

It is proposed that by inhibiting the development of new blood vessels, tumor growth could be terminated. Angiogenesis, the growth of new blood vessels, has been studied over the past 50 years. Anti-angiogenic substances have been proven to prevent vascularization. Recently shark cartilage has been proven to have anti-angiogenic properties. However, no research has been conducted to prove oral consumption of shark cartilage is effective in tumor prevention or reduction. Thus thousands of sharks are slaughtered each year to help support the growth of the shark cartilage industry. This study determines whether oral consumption of shark cartilage has an effect on the development of tumors in mice. Two groups of mice were injected with melanoma cells, with one group receiving shark cartilage mixed with its food. The mice were observed during the study to determine whether the shark cartilage would prevent tumor growth. The results support the hypothesis that shark cartilage orally consumed does not prevent tumor growth. This research hopefully will stimulate further investigations to definitively end the question of the efficacy of shark cartilage as a cancer treatment and prevention.

Introduction

One of the most feared and dangerous predators lurking about the ocean, could save your life. This persuasive headline hailing the benefits of the consumption of shark cartilage has been present in the media over the past ten years. Publications and promotions have stated continuously that sharks don't get cancer. Despite the scientific proof of over 20 cases in which sharks have developed cancer, the myth continues to

thrive (2). Since sharks supposedly don't get cancer there must be a reason for this potential immunity. Dr. Judah Folkman of Harvard Medical School has devoted much time in this research area of cartilage and angiogenesis, new blood vessel growth. Dr. Folkman's research has shown shark cartilage has anti-angiogenic properties (14). However Dr. Folkman does not believe in the efficacy of shark cartilage through oral consumption. Dr. William Lane the author of Sharks Don't Get Cancer and Sharks Still Don't Get Cancer takes a different viewpoint. Dr. Lane has done research in Cuba in which he theorizes, that shark cartilage consumption has had positive results with terminally ill patients. Dr. Lane's books and a *60 Minutes* news segment has been the driving force for the shark cartilage consumption industry. The shark cartilage industry now grosses millions each year, with an estimate of over 100 thousand consumers yearly (5). However, no basic research has been completed to provide concrete evidence on whether oral consumption of shark cartilage has anti-tumor properties. Therefore millions of sharks are killed each year decreasing their population by 80% over the past ten years (5). Since sharks are at the top of the food chain, possible extinction would cause a major ecological imbalance with recovery unlikely due to the age of sexual maturity and low birth rates. It is necessary to determine whether sharks play a vital role in the fight against cancer. If not, we need to stop their annihilation. My research hopes to stimulate further studies which will thoroughly, distinctively, and conclusively put an end to the question.

Background

In 1935, the term "angiogenesis" was first used to describe the formation of new blood vessels. Angiogenesis has become the focus and primary aim of investigations involving solid tumor growth. In 1945, Algire concluded that tumors have a vascular and avascular phase (19). An avascular tumor lacks a network to supply nutrients and remove

wastes. The tumor is in a balanced state with the number of new cells equal to the number of dying cells. According to Dr. Judah Folkman an avascular tumor has never killed a human being (8). For a tumor to become vascular and reach the second phase, it must undergo angiogenesis. Following neovascularization, the malignant tumor is capable of expanding and angiogenesis continues until the host is dead. Dr. Folkman conducted experiments trying to determine what changes a contained group of cells into a malignant tumor. His research concluded that the secretion of diffusable chemical substances called tumor angiogenesis factors, causes the induction of capillary growth (6). Medical approaches to the treatment of malignant tumors were then aimed at finding substances with anti-angiogenic properties. In 1974, Dr. Folkman devised an experimental model using a rabbit's cornea for studying tumor growth and neovascularization (10).

Dr. Folkman considered since cartilage is avascular and using past knowledge hypothesized that bovine cartilage has anti-angiogenic properties. Using his model in 1976, Dr. Folkman isolated a cartilage factor that inhibits tumor neovascularization. This research supported findings of the inhibitory factors of bovine cartilage (14). More research was done in the field using different techniques and variables. In 1983 the realm of shark cartilage was opened. Dr. Lee and Dr. Langer conducted a comparative research study between bovine and shark cartilage (15). They concluded that shark cartilage could have up to 100,000 times more angiogenesis inhibitory activity compared to bovine cartilage. The spotlight was now on the shark. Continued research was conducted trying to identify the substance in shark cartilage that inhibited neovascularization (16). In 1992, Dr. William Lane published his book Sharks Don't Get Cancer which started mass consumption of shark cartilage as a health remedy (12). Dr. Lane in a *60 Minutes* segment claimed his research in Cuba supposedly had significant results on terminally ill patients. However, this research was never published. In 1997, Dr. Paul F. Davis conducted the first study of inhibition of angiogenesis by oral ingestion of powdered shark cartilage (4). A mast cell induced angiogenesis of the mesentery was observed with and without shark

cartilage. This study concluded that there is a direct relationship between inhibition of angiogenesis and consumption of shark cartilage. The most recent study completed in 1998, examined growth and metastatic spread of SCCVII carcinoma with oral consumption of shark cartilage (11). This study showed no connection between the oral administration of shark cartilage and the growth and metastatic spread of the carcinoma. My project will hopefully add to the current knowledge of oral consumption of shark cartilage and stimulate further debate and research.

Hypothesis

The oral consumption of shark cartilage will not affect the growth of cutaneous melanomas in mice.

The possible anti-angiogenic properties of shark cartilage will not work if orally consumed. If prevention of tumor growth occurs then larger studies should be considered. If no correlation to tumor and shark cartilage consumption occurs, then this will be the first study in the United States to disprove the medical use of shark cartilage. Thus stimulating further studies and the possible protection of consumers from the purchase of a product that will not be effective, which is responsible for the depletion of the shark population.

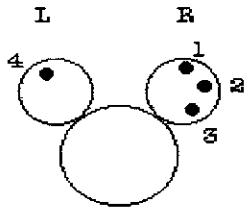
Materials and Methods

All research was conducted at Memorial Sloan Kettering Hospital, New York, New York, from July 26th 1999 to September 1st 1999. The B-16 melanoma cell line was selected as the tumor source. The frozen tumor cells were thawed out in 37^{o}C distilled water. Cells were placed in a T-75 container along with 10 ml of medium. The medium

consisted of 89% MEM, 10% Calf Serum, and 1% Glutamine. Cells were passaged every other day for two weeks, excluding weekends. Trypsin was added to separate the cells from the flask and the cells were centrifuged for 7 minutes. Cells were then placed in fresh medium and stored in an incubator. The cells eventually grew to be placed in two T-175 containers with 25 ml of medium in each.

Memorial Sloan Kettering Hospital mice were used. The mice hybrid type was CB6F1/J. This type of mice is a cross between BALB/cJ male mice and C57BL/6 J female mice. All mice used were females, protocol number 97-11-051. At the start of the experiment they were 1.5 months old.

The B-16 melanoma cells were counted on the day of injection. Two syringes were prepared with 250,000 cells in each. Metofane was used to anesthetize the mice. Mice were placed in a container with the Metofane for 1 minute. Each mouse was pinched to see if a response was present. When each mouse had no response, it was injected with 50,000 cells sub-cutaneously. The mice were injected on the back right side. After injection the mice were ear marked for identification purposes. The marking tool was a clipper and the key for the mice ear marks is shown below:



The mice were separated into two groups. The 5 mice in the experimental group and 5 mice in the control groups were noted by identification on their cages. Both groups of mice were weighed using an electronic scale and weights were recorded. All food was removed from the mice cages.

In the control group *Square Meal Food* produced by *L/M Animal Farms* was placed into the food holder. Also within the cage was a filled water bottle. The level of food was kept constant throughout the study.

In the experimental group all food was removed that was previously in the cage. The experimental group received food in non spill dishes that were placed securely in the cage. Each dish held 12 g of food. The food placed within the dish was a mixture of shark cartilage and ground *Square Meal*. The shark cartilage used was BeneFin, a product of Lane Labs. It is organically processed and is a 100% shark cartilage, with no additives. The dish consisted of 8 g of BeneFin shark cartilage and 4 g of *Square Meal*. This made the ratio of BeneFin shark cartilage to *Square Meal* 2 : 1. The mice were given as many dishes as their appetites demanded. The dosage which the experimental mice received over the course of the study was 3.71 g/kg per mouse per day. This can be compared to the suggested dosage for humans which is approximately 0.1 g/kg per day.

The mice were observed and evaluated. The food was refilled in the control group. In the experimental group 6 more dishes of the shark cartilage combination were placed in the appropriate cage. The water bottles were monitored and kept filled. Each time the mice were weighed and evaluated to detect tumor growth. Observations and data were recorded accordingly for a 26 day duration. After the 26 day treatment period, mice were given to Memorial Sloan Kettering Hospital for additional purposes.

Results

Fourteen days post injection of melanoma cells, tumor growth was observed in one mouse in the control group. On day 17, tumor growth was noted on a mouse fed shark cartilage.

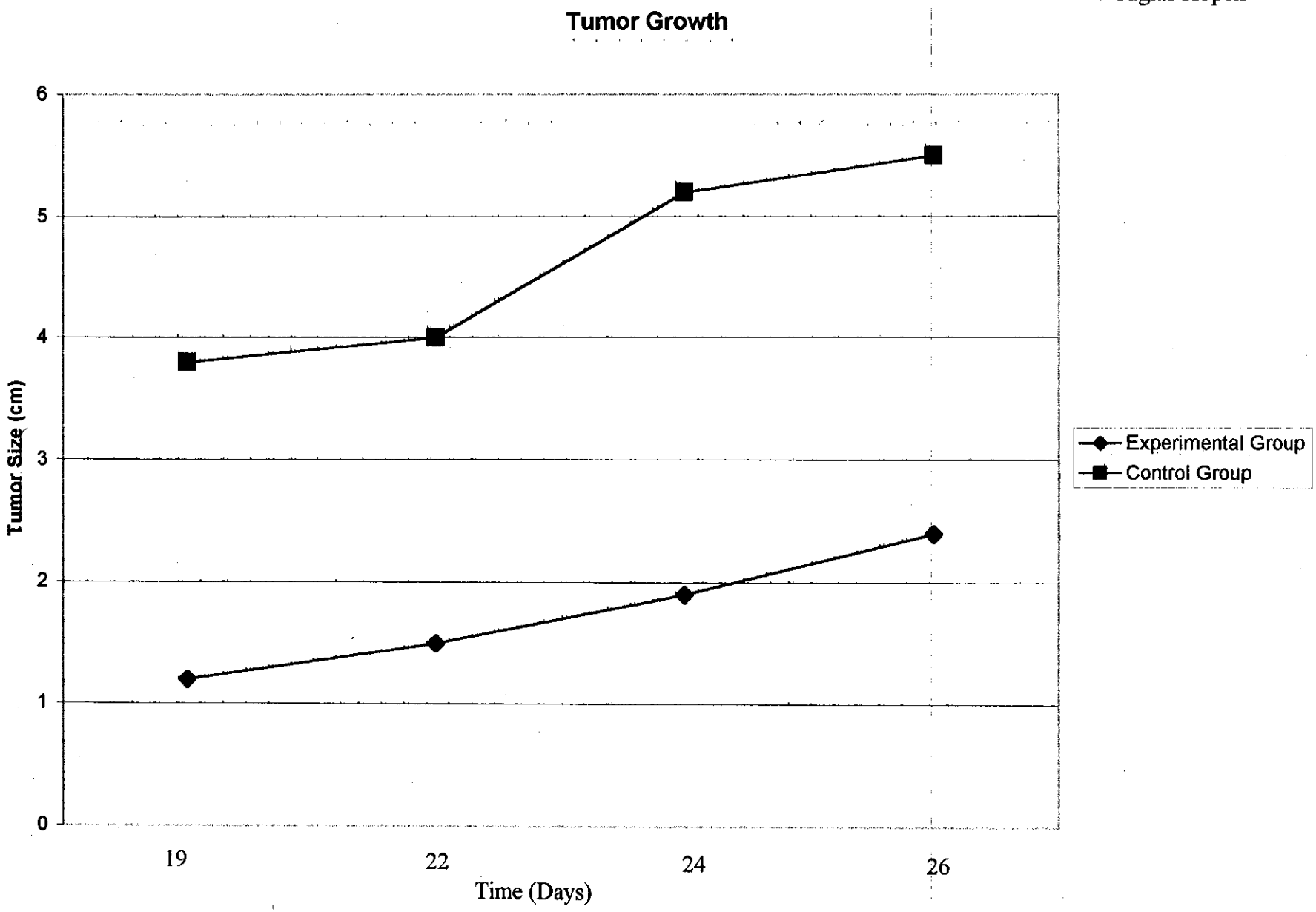
Tumor growth was measured using a mouse tumor measuring device. The instrument measured the diameter of the tumors. In the control group tumor growth increased significantly over time. In the experimental group, tumor size doubled by the end of the study. The size of the tumor in the control group was greater than those in the experimental group. In both groups the tumors continued to enlarge as time progressed.

The weight of the mice throughout the study was measured. The average weight of the control group was greater than the shark cartilage consumption group. The shark cartilage consumption group despite having lower values, maintained their body weight. After tumor growth the weight of a mouse would subsequently increase.

In chart 1, the tumor size of the experimental mouse and control mouse are graphically shown.

Chart 1

Douglas Hopek



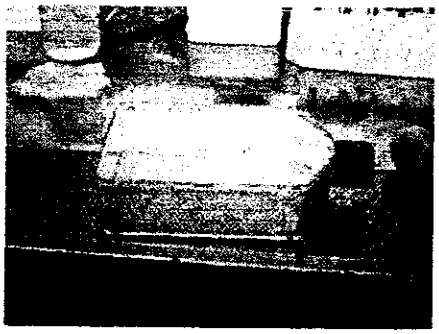


Figure 1

Figure 1 shows a picture of the flask holding the medium and melanoma cells on the day of injection.

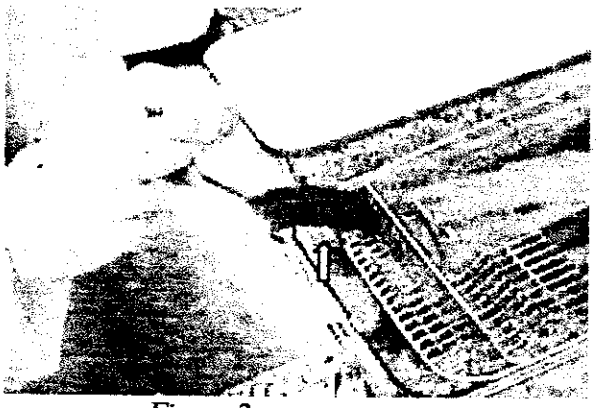


Figure 2

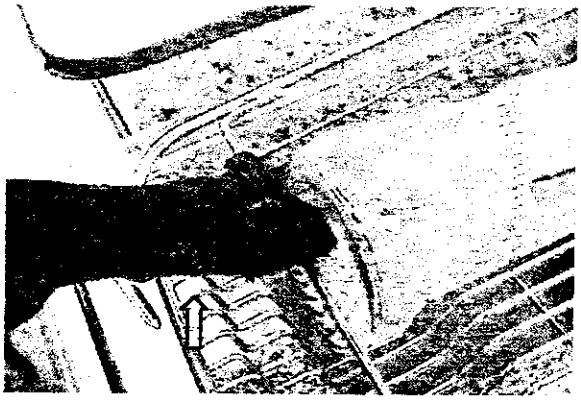


Figure 3

Figures 2 and 3 show pictures of mice that have developed tumors. The red arrows point to the tumor growth. The mouse in figure 2 is from the control group and figure 3 is the mouse from the shark cartilage group.

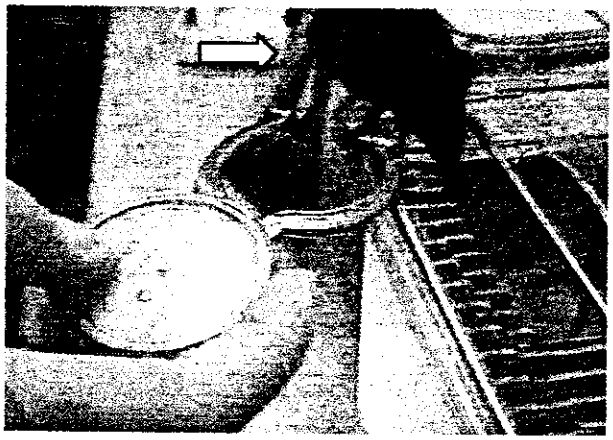


Figure 4

The photograph in figure 4 was taken on the final day. The tumor growth of the control group mouse was fairly large and is indicated by the red arrow. The instrument shown is the device used to measure the tumor growth.

Discussion

In the study reported here, it was hypothesized that oral consumption of shark cartilage would not have an affect on melanoma growth in mice. Even with a dosage of shark cartilage 40 times that of the human recommendation, tumor growth was observed proving the hypothesis.

The number of mice in the study was small and if no growth or growth in only in the control or shark cartilage group occurred, then the conclusions would be debatable. But with growth in both groups the results are significant. In the study not all the mice in the control group developed tumors. This would negate the theory that the mice that did not develop tumors in the experimental group were protected by the shark cartilage. The reasons for non development could be the number of melanoma cells, age of mice, and the genetics of the individual mice. If sharks do not get cancer then shouldn't the consumption of shark cartilage work each and every time? There seems to be a little doubt that anti-angiogenic therapy for tumor growth such as angiostatin and endostatin will have a place in the arsenal of cancer therapy, but the consumption of shark cartilage will not.

Very few studies on oral consumption of shark cartilage and its effects on tumor growth have been published in the world. Hopefully this research project might act as a stimulant to spear on research to re-verify these results and definitely end the debates on shark cartilage. It would then be possible to create a public awareness of the falsehood of shark cartilage as a cancer treatment. In doing so, consumers would be saved from needless spending of millions of dollars each year. More importantly, the pressure upon the shark population would be eased, and the threat of an ecological imbalance would be alleviated, thus leaving the world a better place.

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