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EXPERIMENTAL USE OF CRYOSURGERY TO TREAT FIBROPAPILLOMAS IN THE GREEN TURTLE, *Chelonia mydas*

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INTRODUCTION

The occurrence of fibropapillomas in the green turtle, *Chelonia mydas*, has been well documented in the Hawaiian Islands, Florida and elsewhere worldwide. The incidence of these tumors and subsequent mortality in Hawaii has increased dramatically over recent years. Numerous theories have been advanced regarding the causative agent, i.e., parasites, viruses, environmental pollutants. However, the etiology of these tumors remains to be determined (Balazs and Pooley 1991).

Kaneohe Bay on the Island of Oahu, Hawaii, harbors a large population of immature and adult turtles. The first known case of fibropapillomas at this location was confirmed in 1958. Currently, 50-90% of these turtles are found to have tumors, depending on the study site sampled in Kaneohe Bay. Strandings of emaciated, weak and/or dead tumored turtles have been increasing throughout the Hawaiian Islands. Many turtles survive lengthy periods with severe tumor infections. Death usually occurs when tumors obstruct vision, become extensive in the mouth or throat area, or affect the internal organs (Balazs 1991).

This study was initiated to determine if cryosurgery was a viable method of treating fibropapillomas for turtle rehabilitation and release, and also for practical use in field treatment and release.

BACKGROUND OF CRYOSURGERY

Cryosurgery is the application of cold to tissue resulting in the destruction of that tissue. The concept is not new. An Englishman is credited for the first use of an ice-cold brine for the treatment of human breast and skin cancer in 1851. In 1961, liquid nitrogen was first used in human medicine followed by its use in veterinary medicine in the 1970's (Hoyt and Seim 1981). The main use of cryosurgery is for the removal of both malignant and benign tumors.

Several different types of refrigerant are generally used. Liquid nitrogen, which reaches a temperature of -195.8° C (-320.5° F) is the most desirable. Nitrous oxide, which reaches a temperature of -89° C, can also be used. Nitrous oxide is generally used with a probe and confined to small tumors less than 1 cm in diameter. This study used liquid nitrogen as the refrigerant.

Cell death usually results from two applications of a refrigerant that cools the cell temperatures to -15° C to -20° C. Optimal results are obtained with a rapid freeze and a thaw to room temperature. Three or four freeze thaw cycles may be indicated for large or very dense tumors.

The mechanisms of cell death are accomplished in several stages (Seim 1980):

Cell Dehydration - Ice crystals are formed in the extra cellular fluids resulting in a hyperosmolar condition. Fluids flow out of the cells, resulting in cell death from high concentrations of electrolytes.

Ice Crystal Formation - Ice crystals also form within the cells. These crystals melt during a slow thaw and recrystallize forming larger crystals that damage and rupture the cell walls. This condition does not occur during a rapid thaw.

Protein Denaturation - The lipid-protein complexes within the cells are denatured, thereby damaging the cell membrane.

Thermal Shock - The direct result of rapid low temperature change by itself damages the cells.

Delayed Phase - Occurs several hours after the freeze cycle, and is related to vascular restriction of blood flow.

Besides cell death with resulting tumor death and disappearance, cryosurgery may have additional benefits. Human tumor cells can induce specific tumor antigens which can in turn stimulate the immune system to produce cytotoxic tumor cells. These cells in turn destroy additional tumor cells. Cryosurgery of tumors in mice stimulated an immunological response, thereby, resulting in slower and/or reduced tumor formation (Neel 1980). Such an effect remains to be demonstrated with fibropapillomas in the green turtle, but would represent a significant finding if it occurred.

MATERIALS AND METHODS

The basic cryosurgical materials consist of:
Liquid nitrogen storage container - These containers vary in size from 10 - 50 liters. The liquid can be stored for one month or longer, depending on the type. It is possible to obtain such units from cattle breeding associations that handle semen.

Hand held cryosurgical spray containers - They are available from several manufacturers and come with various interchangeable tips that vary the amount of liquid and vaporized nitrogen to be sprayed on the tumor.

Probes - These are metal tips placed directly on or in the tumor growth. Nitrogen spray is circulated through the probe where it is super cooled and vented through a tube. Probes are used on small tumors (1 cm or less) and around areas such as the eyes where excessive spraying of nitrogen may damage normal tissue.

A Temperature Monitor - This consists of a temperature gauge with one or two needle thermocouples. The needles are placed in various locations to ensure proper freezing temperatures are reached.

Liquid Nitrogen - This may be purchased through a cattle breeders association or a supplier of oxygen or welding gas.

A green turtle with extensive tumors was selected for cryosurgical study. This turtle had a carapace length of 54.7 cm and weighed 19.5 kg. A total of 62 tumors were present ranging in length from less than 1 cm to greater than 10 cm. Seven different tumors were selected for freezing, ranging in total length from 1 cm to 6 cm. The locations were: left eyelid, right eyelid, neck, shell, left front flipper and left rear flipper. A lidocaine nerve block was injected at the base of the tumors to eliminate pain. This was especially important for the eyelid tumors, as this area was very sensitive to the touch.

Temperature monitoring needles were used in most of the tumors to assure proper freezing levels. It was difficult to use the monitor in certain areas around the eyes because of the sensitivity of the area and difficulty in keeping the needles in place. When the probes were not used, the amount of freeze was monitored by visual observation of the ice ball and digital palpation. Two freeze cycles were employed using the rapid freeze, room temperature thaw technique.

The turtle was maintained in a sea water tank and the results monitored by photographs every week until tissue necrosis was complete. After approximately four months, the turtle was released into the wild.

An additional turtle was captured in Kaneohe Bay, Oahu, with a carapace length of 56.6 cm and weight of 30.4 kg. Two tumors were frozen (one eyelid tumor and one front flipper tumor) and the turtle was released. This turtle was recovered approximately five months later and the results evaluated by photography.

RESULTS

Tumors up to 5 cm in length could be frozen successfully with the hand held spray container. The general sequence of post-freezing tumor necrosis in the green turtle was:

Immediate - Swelling and erythremia of tissue 1 - 2 hours post freeze.

One week - Tumor appeared soft and jelly-like with little loss of tissue.

Two weeks - Some necrosis of tissue started, but not extensive.

Three weeks - Loss of tumor tissue clearly evident.

Four weeks - Necrosis of tissue continuing. Loss of one-half of tissue may be evident.

Five weeks - Tumor necrosis might have been completed without evidence of scar formation. Other tumors only appear three-quarters complete.

Eight weeks - Tissue necrosis complete. Scar formation evident.

Eyelid tumors seemed to be more resistant to freezing. These two tumors had to be refrozen because of persisting tumor tissue after two months. The growths were smaller, but not significantly reduced in size. This "resistance" may be due to incomplete freezing temperatures resulting from difficulty in using the temperature monitor and the fear of freezing vital eye structures.

The result of the field freeze in the one turtle was similar to the experimental captive turtle. The tumor lesion was completely healed and gone after five months. The eyelid tumor did not appear any larger, but appeared to be about the same size after five months.

SUMMARY OF ADVANTAGES AND DISADVANTAGES OF FIBROPAPILLOMA CRYOSURGERY

Advantages:
Equipment can be used for field treatment.

Procedure can be used on large (5 cm) growths as well as small growths.

Very little bleeding or trauma involved.

Procedure may produce an immunologic effect, thereby reducing additional tumor formation.
Treatment is "cost effective".

Disadvantages:

Eyelid tumors, which are the most damaging to the turtle, may require additional freezes or thermal monitoring.

Some experience is required by the cryosurgeon.

Must use care around vital structures such as the eyes.

Basic cause of tumors not corrected. Regrowth may occur. Long term (years) benefits need to be evaluated.

DISCUSSION

Several interesting observations resulted from the study. The total time from the initial freeze until complete tissue necrosis and scar tissue formation was longer than that in domestic animals. In canine and feline fibropapilloma-type tumors, tissue necrosis is complete in 1 - 2 weeks. Complete healing with scar formation occurs in 3 - 6 weeks (Goldstein and Hess 1976). In this study 5 - 8 weeks were required for complete tissue necrosis. Perhaps sea water retarded the separation and dehydration of the dead tissue.

The use of cryosurgery for the treatment of ocular squamous cell carcinoma in cattle resulted in a 95% cure rate. It was stressed that the lowest cure rate occurred when temperature monitors were not used (Farris and Fraunfelder 1976). This may be the cause of the lack of success with the green turtle eyelid tumors. Smaller needle monitors may be indicated.

The use of cryosurgery in the treatment for green turtle fibropapillomas shows promise. The method can be used in rehabilitation centers and in field tag and release projects. The freezing of small "starting" tumors as well as growths up to 5 cm may be lifesaving for many turtles.

Additional trials in captivity are needed in order to properly evaluate the possible occurrence of cryosurgery stimulating the immune system to destroy other tumor cells.

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