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Extract of Pine Cones Augments Tumor Response to Electrochemotherapy

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The application of direct current pulses to living cells can cause a temporary membrane destabilization which has been termed electroporation. The primary effect of electric pulses on cells is that when a destabilized state is induced, it is possible for molecules that are normally nonpermeant to enter the cytoplasm of cells. This effect has become part of procedures for monoclonal antibody production, cell-cell fusion, cell-tissue fusion, membrane protein insertion, and genetic transformations. The effect has also been exploited to locally deliver chemotherapeutic agents to tumor cells by administering a drug and then locally applying electric pulses. This treatment, known as electrochemotherapy (ECT), has been used successfully in animal models for many types of tumors and in clinical trials in the US and Europe. ECT typically provides rapid destruction of treated tumors (within days) in animals and in the clinic. Tumor recurrence rates after ECT vary with the animal model/tumor type. In order to reduce recurrences, this study combined ECT with pine cone extract (PCE) in order to assess effects of this adjuvant treatment on the recurrence rates of tumors in a challenging animal model for melanoma.

Pine cone extract has a medicinal history that dates back to the Greek physician Dioscorides (A.D. 514). Interest in complementary and alternative medicines in the past 20 years has led to compelling evidence to suggest that PCE has very strong immunologic effects. Thus, the motivation for combining ECT and PCE was to capitalize on any immunologic effects in host animals to reduce recurrence rates. Extract was prepared for this study as an aqueous alkaline solution containing lignins (polyphenylpropenoid) complexed with polysaccharides (Sakagami et al, Chem. Pharm. Bull. 38(11) 3031-3034, 1990). The active component(s) in PCE are not completely understood but are thought to be complexes with molecular weights greater than 100 kDa. They are resistant to alkaline conditions, autoclaving, treatment with proteases, and nucleases but are susceptible to treatments that specifically degrade the lignin and/or polysaccharide components.

The tumor model was established by injecting 1×10^6 cultured B16F10 murine melanoma cells (American Type Culture Collection CRL-6475) into the flanks of female C57Bl/6 mice. Tumors measuring about 5 mm in diameter resulted after 7 days of growth in the animals. Electrochemotherapy treatment was conducted by injecting a 50 μ l volume of bleomycin (4 Units/ml) into each tumor and

then applying a series of six 100 μ s DC pulses to the tumor. The applied field strength of each pulse was 1500 V/cm. Animals were then returned to normal housing and followed for tumor regression and subsequent recurrence for a period of 50 days after ECT treatment. Two different PCE administration routes were used to determine the effects of the extract on the ECT treated mice. The first was the addition of 4 concentrations of PCE to the drinking water ranging from 100 μ g/ml to 1000 μ g/ml. Animals were provided PCE for the entire 50 day study period. The second administration route was by injection. Mice received intratumor injections of 1, 2, 5, or 10 ng for five consecutive days starting the day of ECT treatment.

Results were quantified with respect to the number of animals that responded completely to their treatment which was defined as the complete absence of tumor. Groups of animals that received oral PCE after electrochemotherapy had complete response rates ranging from 50 to 64 percent 50 days after initial treatment. In contrast, animals in these same experiments that received only ECT had a 31 percent complete response rate. This represents a doubling of the complete response rate attributed to PCE. Similarly, animals treated with intratumoral PCE after ECT had complete response rates ranging from 81.8 to 100 percent. Animals treated with ECT alone at the same time has a 25 percent complete response. Thus intratumoral PCE administered for 5 days post treatment increased the complete response rate at day 50 by 3 to 4 fold.

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