The Use of Plants in Predicting Cancer Inhibitor Effects in an Animal Neoplasm

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INTRODUCTION

In cancer research today, a great deal of work is being done to determine the chemotherapeutic value of various cancer control agents in animal malignancies. In this project I explored the possibility of using plant neoplasms to predict anti-cancer drug effectiveness and mode of action in animal cancers.

This study showed that the plant growth used could predict cancer control drug effects in animals with an accuracy of 77%, and that drug mode of action trends were generally similar in the animal cancer and plant growth. This indicates that plants could possibly yield general data on anti-cancer drug effects in animal cancer. The accuracy of prediction using a plant system was limited by variation in metabolism in plants and animals, organism reaction to cancer invasion, and drug effects in different cancer types.

The selection of cancer-inhibiting drugs was from the experimental cancer drugs currently in the research stages.

EXPERIMENTAL PROCEDURES AND MATERIALS

In order to produce valid results, it was necessary to conduct a broad range of experiments. The purpose was to explore the feasibility of using plant growths to predict cancer control drug effects on animal neoplasms. The cancer control agents used in this project were: 6-mercaptopurine, 6-chloropurine, and 8-azaguanine. It has been found that 6-mercaptopurine is highly effective in reducing neoplasms in animals; 6-chloropurine has shown some effectiveness in retarding animal neoplasmic growth; and 8-azaguanine has indicated very little success in halting animal cancer. Experiments will be described in the order that they were performed.

I.

Series A

These experiments involved the developing of 2,4-D calluses on bean stem sections using concentrations of 2,4-D at 10^{-5}M, 10^{-6}M, 10^{-7}M. Groups of five bean sections, 18 mm in length, were placed in the concentrations of 2,4-D; all sections were sterilized in a 1:10 *Chlorox* solution, and placed basal end down in the varying concentrations of 2,4-D. The weights of the bean stem sections were computed before and after the application of 2,4-D. The exposure times for each concentration were as follows: 10^{-5}M, - twenty-four hours; 10^{-6}M, - seventy-two hours; 10^{-7}M, - one hundred twenty hours. Data were recorded at two-day intervals as was the case in all bean stem experiments.

Series B

In this series of experiments, the cancer control agents listed above were prepared in 10^{-3}M, 10^{-5}M and 10^{-7}M concentrations. Equal amounts of each cancer control drug concentration were mixed with an equal volume of 2,4-D of 10^{-7}M concentration. This series of experiments showed the effect of the cancer control agent when applied in varying concentrations on the early stages of plant growth formation. Groups of five bean stem sections were exposed to each concentration of cancer control agent plus 2,4-D of 10^{-7}M concentration. The weights of the sections were computed before and after application.

Series C

In these experiments, 2,4-D calluses were induced on bean stem sections with a concentration of 10^{-3}M of 2,4-D; after callus formation the cancer control agents were applied in concentrations of 10^{-3}M, 10^{-5}M, and 10^{-7}M. Groups of five bean stem sections with calluses were exposed to each concentration of cancer control drug. The weights of the bean sections were determined before and after the application of the cancer control drugs in their various concentrations. The exposure time for each concentration of cancer inhibitor was: 10^{-3}M, 24 hr; 10^{-5}M, 72 hr; 10^{-7}M, 120 hr.

II.

Series A

In these experiments, crown gall tumors were allowed to fully develop prior to the application of the cancer control agents at a concentration of 10^{-3}M. It was felt this experiment would supply information on the effectiveness of the anti-cancer agents used. Three lots of five carrot discs were allowed to develop crown gall tumors and then were treated with the cancer control drugs. The exposure time for each cancer control agent 10^{-3}M was 24 hours. Data were recorded at three-day intervals, as was the procedure for all carrot disc experiments.

Series B

In these experiments, the cancer agents: 6-mercaptopurine, 6-chloropurine, and 8-azaguanine were applied in the concentration 10^{-3} at the time of inoculation with *Agrobacterium tumefaciens*. This series of experiments predicted cancer control drug effects when applied at the early stages of crown gall formation. Groups of five carrot discs were exposed to each cancer control agent.

Series C

In these experiments, crown gall tumors were allowed to develop on the carrot disc. This series of experiments predicted a control for series A and B on carrot disc experiments.

III.

Series A

In this group of experiments, the crown gall causing organism, *Agrobacterium tumefaciens*, was grown on a sterile medium and the cancer control agents were administered by *in vitro* technique. It was felt that these experiments would provide information on cancer control drug effects when applied to *Agrobacterium tumefaciens* in a pure culture. Equal volumes of the medium and cancer control agents in the concentration of 10^{-3}M were mixed and autoclaved. These experiments lasted

three days. Bacteria counts were made under dark field illumination to determine cancer drug effectiveness.

Series B

Series B served as a control for series A. A bacteria count was made and the same procedures followed as in series A.

IV.

Series A

In these experiments, crown gall tumors were allowed to develop in coleus, *Pepperonia,* geranium, and carrot, and were treated with the cancer control agents of 10^{-3}M concentration. These experiments gave data on the effectiveness of the cancer control agents in retarding the spread of the crown gall tumors. This experiment lasted two weeks.

EXPERIMENTAL PROCEDURE IN ANIMALS

It was necessary to conduct the animal experiments in order to correlate plant results. The experimental animal in this study was the Swiss albino mouse; each specimen weighing from 18-20 grams. The malignancy experimented with in this project was the Ehrlick ascites tumor. This malignancy grows as a liquid mass in the abdominal region of the white Swiss albino mouse. This tumor was chosen because of its ability to yield very accurate results of factors that influence it and its ability to be transplanted from one mouse to another. It might be pointed out that this malignancy has never been found in man; however, it has been useful in determining the effectiveness of cancer-inhibiting agents on some human tumors. The ascites is usually fatal to the Swiss albino, but every effort was made to prolong the life of the experimental mice. The experiments conducted on the Swiss albino were:

Series A

The application of 30 mg/kg of mouse weight was given at the time of ascites transfer into the mice. It was hoped this experiment would show the effectiveness of the drug in retarding early stages of tumor formation. This experiment lasted for ten days.

Series B

This involved the application of the cancer control agents in the following doses: 40 mg/kg, 30 mg/kg, 20 mg/kg, and 15 mg/kg; each applied daily for a three-week period.

Series C

In this experiment, the ascites tumor was allowed to develop uninhibited in order to provide a means of control for mice experiments. The weight of each mouse was taken before and after the application of cancer control agents in all animal experiments; all conditions were aseptic. Series C experiments lasted eight days.

Microscopic examination was made of treated and untreated plant neoplasms; likewise with treated and untreated animal Ehrlich ascites tumor. The microscopic examination was made to determine the results of the anti-cancer agents in the malignant cells. Results concerning these observations of plant and animal growths were recorded. It was necessary that slides of the plant and growths be prepared. Plant slides were much more difficult to make than the animal slides. This presents a problem in using a plant system to predict cancer control effects in animal cancers.

Results and Discussion

Experimentation and microscopic examination were employed in an effort to determine cancer control drug effectiveness and trends of action. The chemotherapeutic value of the cancer control agents were expressed in percent. These percentages were computed after careful visual and photographic study; since the emphasis in this project was on final results, only the final data will be discussed. The comparison between plant neoplasms and animal cancer has been mentioned.

(The theories put forth here about drug mode of action are those of Dr. Anton Lindner and Dr. G. A. LePage. Their methods were applied to plant tissues to see if their results might be duplicated to some extent in a plant neoplasm. These scientists found that 6-mercaptopurine and 6-chloropurine affected the synthesis of the cellular nucleic acid base, purine. They showed that with 8-azabuanine the conversion of the purines adenine to guanine in DNA was inhibited.)

Upon microscropic examination of the plant neoplasmic cells treated with 6-mercaptopurine and 6-chloropurine, it was found that there was evidence of nuclear distintegration. This could be indicative of DNA decrease, perhaps brought about by lack of purine for DNA formation. The same characteristics were noted in the animal cells treated with 6-chloropurine and 6-mercaptopurine, where it was known that these two compounds would inhibit purine synthesis. It is assumed that the effect in the animal was the same as that in the plant. Eight-azaguanine showed the same effect of nuclear disintegration. The treated cells in both plant and animal growths showed the same characteristic drug effects in this case, leading to the postulation that the effect of 8-azaguanine on the plant growth was the same as that on the animal growth.

Of the plants used, *Pepperonia* and carrot appeared to be the most accurate for the purpose of determining cancer drug effectiveness.

Conclusions

Animals and plants were treated with the same cancer control agents, and comparisons were made; they led to the following conclusions:

1. The results of Lindner and LePage are believed to have been duplicated in plants.

2. The effectiveness of cancer control agents was nearly the same in an animal as in plants.

3. 8-Azaguanine and 6-chloropurine are indicated by experimentation to warrant no further study.

4. The effectiveness of 6-mercaptopurine in retarding crown gall or Ehrlich ascites does warrant further study.

5. All microscopic examination supports the findings of Lindner and LePage in plants and animals.

This project has dealt with only a very small area of the cancer treatment problem. More research is needed on the use of plants to predict the chemotherapeutic value of cancer control agents in other animal malignancies.

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