Comparison of the Effect of Three Nucleosides and a Liver Emulsion Upon the Inhibition of Cancer in Mice

LEONELL C. STRONG, PH.D.
and HENRY MATSUNAGA, B.S.*

The sum total of inhibition of growth capacities of spontaneous tumors in mice obtained with three nucleosides used separately is similar to, if not identical, with the amount of inhibition obtained by the use of the original liver emulsion. There is also clear evidence that most of this tumor inhibition is brought about by 5 methyl cytidine (0.02 molar concentration administered intraperitoneally three times weekly with a maximum injection of a volume of 0.05 cc). Adenosine is not only an inhibitor of cancer in mice but, under certain conditions, also a stimulator (0.05 molar percentage solution with maximal injections at any one time of 0.05 cc volume). This conversion of an inhibitor of cancer to a stimulator has been brought about by aging of the solution but how the conversion takes place is not known. However, this conversion of the effect of adenosine was obtained by the same procedure that the inhibitor of cancer in the liver emulsion can be converted to a stimulator of cancer.

INTRODUCTION

It has been recorded, "that three nucleosides that are known to be present in a liver extract which has an inhibitory effect upon the growth of spontaneous tumours of mammary gland origin in mice, have been tested upon this same

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*The authors are from The Leonell C. Strong Research Foundation, Inc., 10457-I Roselle Street, San Diego, California 92121. The first author is Director of the foundation.

type of cancer. They have been tested singly and, two of them, in combination with each other, and in the same molar percentage as they occur in the original liver emulsion. It has been shown (1) that a 0.05 molar percentage solution of adenosine may serve as either an inhibitor or a stimulator of cancer depending upon the age of the solution, (2) that an 0.08 molar percentage solution of 6 methyl adenosine is also an inhibitor of cancer, (3) that a 0.02 molar percentage solution of 5 methyl cytidine is the best inhibitor of the three nucleosides and (4) that a combination of a 0.05 molar preparation of adenosine and an 0.08 molar solution of a 6 methyl adenosine will stimulate the growth of cancer in mice. Thus the similarity of effect of these three nucleosides upon the growth of cancer as compared to that by the original liver extract is striking." (Strong and Matsunaga, 1970)

These observations on the effect of three nucleosides on the growth of spontaneous tumors in mice were made on mice of the C_3H/ST strain of the 0 class in which there had been no ancestor of the cancer proband that had received the liver emulsion.

The purpose of the present paper is to present evidence that the sum total of inhibition of cancer growth in mice obtained by using, separately, solutions of these three nucleosides (a) adenosine, (b) 6 methyl adenosine and (c) 5 methyl cytidine is very similar to, if not identical, with that obtained with the original liver emulsion.

MATERIALS AND METHODS

All mice used in this investigation bore spontaneous adenocarcinomata of mammary gland origin. They belonged to the well known C_3H/ST and C_3 HB/ST strains that have been the source of spontaneous tumors for the present series of papers dealing with the inhibition and control of these neoplasms. In this communication, in order to analyze the observations of inhibition of spontaneous tumors with the nucleosides more in line with that obtained with the liver emulsion, all classes of genetic descent of the cancer proband have been included. These classes are (1) 0, in which there has been no treatment with the liver emulsion into any ancestor of the cancer proband (2) E, in which the mother of the cancer proband had received the liver emulsion and (3) 0.01, (4) 0.001, (5) 0.0001, etc. in which (3) the grandmother had received the liver emulsion (4) the great grandmother 0.001 and (5) the great, great grandmother 0.0001, etc. Further, some cancer probands had had ancestors in which one had received the liver emulsion and some had had 2 or more ancestors with liver treatment. Thus the "transmissible entity" indicated by previous work in taking into account the manner in which the cancer proband's maternal ancestors had experienced an injection of the liver emulsion, must now be considered in the analysis of the mechanism involved in cancer control—and

thus the present comparison of nucleoside effect on cancer control with the liver emulsion effect may become more significant.

The nucleosides were made up in distilled water in the same molar concentration as they were found in the liver emulsion. These are (1) adenosine 0.05 molar percentage, (2) 6 methyl adenosine 0.08 molar percentage and (3) 5 methyl cytidine 0.02 molar percentage (Mittelman). They were placed in rubber stoppered serum bottles, which were wrapped in aluminum foil, and kept in the refrigerator at 2.2°C when not in use.

Before injection into mice, the solutions were warmed to room temperature and injected intraperitoneally into mice following the measurement of tumors. The solutions were injected at room temperature in order to avoid spasms which usually follow the intraperitoneal injection of refrigerated material.

The tumors were measured in the two longest diameters three times weekly. These values were multiplied together in order to estimate their relative size.

RESULTS

The data on (1) growth rates of tumors in mice for all three nucleosides and (2) the average increments of growth at the 25th observation period for the mice receiving adenosine alone are plotted on two figures. In Fig. 1 are shown the average growth rates of tumors for (1) the controls on the solid line solid dot curve (2) the mice receiving 6 methyl adenosine on the short dash open circle line (3) the mice given adenosine on the long dash open circle line (4) the mice receiving the liver emulsion on the solid line (5) the sum total of the three groups of mice receiving one of the nucleoside are on the dot and short dash curve and (6) the mice receiving 5 methyl cytidine are on the solid open circle curve.

Figure 2 presents the average increments of growth of spontaneous tumors for four series of mice at the twenty fifth observation period receiving (1) adenosine for the four successive age groups at the open circle points and (2) the controls at the solid dot point. Thus in three successive series, adenosine was an inhibitor of cancer whereas at a later date adenosine stimulated the growth of cancer.

DISCUSSION

Taking into consideration the data on the growth rates of spontaneous tumors occurring in mice of all classes of individuals based upon the liver emulsion treatment in one or more ancestors of the cancer proband, it would seem that all three nucleosides will inhibit spontaneous cancer to a certain extent.

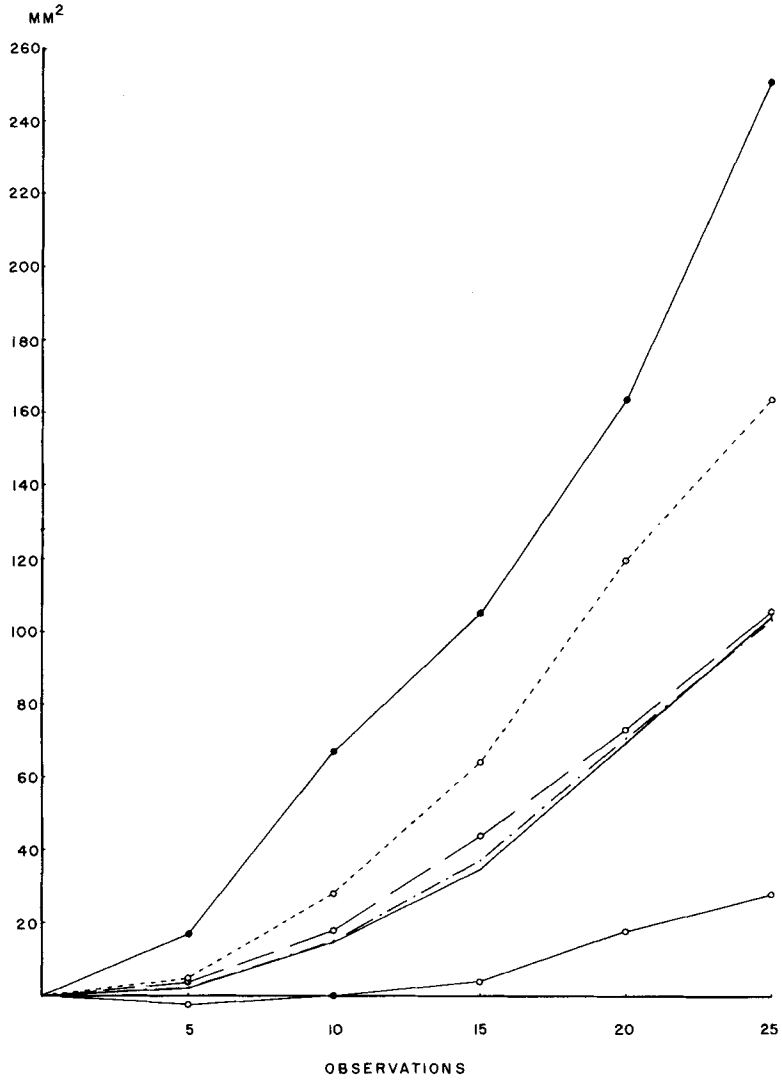

Fig. 1. The data on growth rates of spontaneous tumors for (1) the controls on the solid dot solid line, (2) the mice receiving 6 methyl adenosine on the short dash open circle line, (3) the mice given adenosine on the long dash open circle line, (4) the mice receiving the liver emulsion on the solid line, (5) the sum total of the three groups receiving one of the nucleosides are on the dot and short dash line, and (6) the mice receiving the 5 methyl cytidine are on the solid line and open circle curve. Abscissa: time expressed in periods of observations at the 5th, 10th, 15th, etc. periods. Ordinate: the average increment of growth expressed in mm^2.

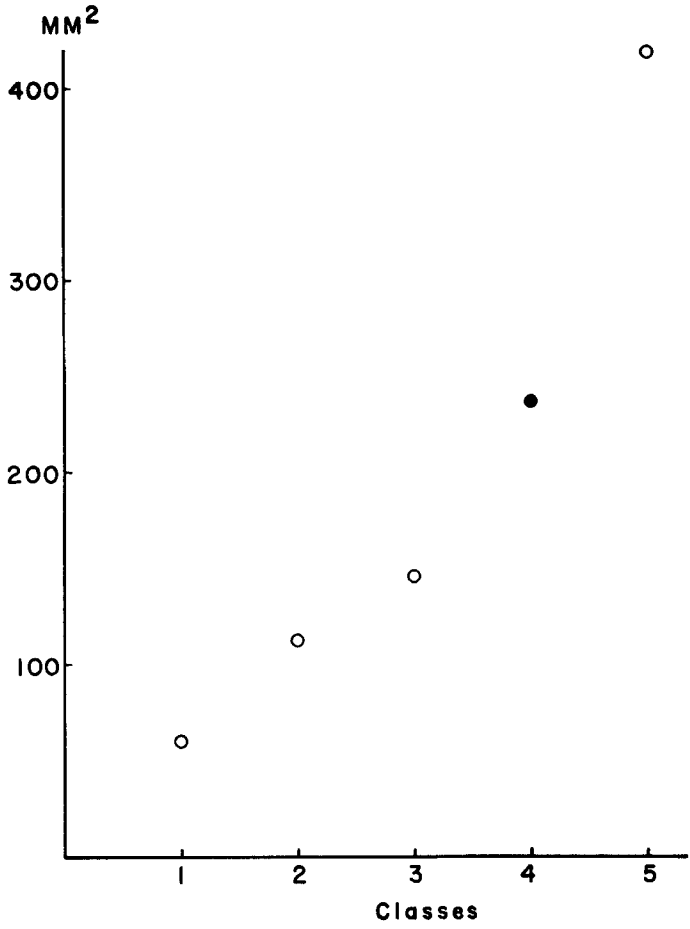

Fig. 2. The average increments of growth of spontaneous tumors for four series of mice at the twenty-fifth observation period receiving (1) adenosine for the four successive age groups at the open circle points and (2) the controls at the solid dot point. Abscissa: separate series of mice. Ordinate: Average increments at 25th observation period in mm^2.

All three nucleosides show values of inhibition of cancer when compared to the controls (solid line solid dot curve of Fig. 1). In sequence of effectiveness in suppressing cancer these nucleosides may be listed as (1) 6 methyl adenosine (2) adenosine and finally (3) 5 methyl cytidine. Of these, the effect of 6 methyl adenosine is halfway between the controls and that obtained with the liver emulsion. The effect of inhibition with adenosine is slightly less than that obtained with the liver emulsion. The inhibition of cancer with 5 methyl cytidine is, by far, greater than that obtained with either of the other two nucleosides. It is also obvious that the average growth rate of spontaneous tumors obtained by the data with the three nucleosides added together (the dot and short dash

line of Fig. 1) is nearly identical to the growth rate of cancer as obtained by the original liver emulsion used alone (solid line of Fig. 1).

Some difficulty of interpretation is, however, encountered when the concept of a "transmissible entity" is taken into consideration in the mechanism for the control of the growth of a spontaneous tumor. This was not a factor in a previous experiment in which evidence was reported that all three nucleosides were tumor inhibitors since only mice of the 0 class, in which no treatment of liver emulsion into any cancer proband's ancestor had been used (Strong and Matsunaga, 1970). It has been shown that the effectiveness of the "transmissible entity" to suppress the growth capacity of the spontaneous tumor is increased in successive generations (between the 0 and the 0^6 generations of mice exposed to a liver injection).

The "transmissible entity" in relation to the control of cancer in mice may have been initiated originally by the injection of the liver emulsion. The evidence bearing upon this concept is not too clear. All that can be said with certainty is that the "transmissible entity" was detected following the injection of the liver emulsion into mice over a number of generations and that now it is sensitive to (1) the further injection of the liver emulsion intraperitoneally and (2) to the presence of neomycin in the drinking water of the tumor bearing mouse. With the division of the spontaneous tumor bearing mice into classes determined by relation to the injection of the liver emulsion into one or more ancestors of the cancer proband, the final analysis cannot, at present be made. With the present cost of some of the pure nucleosides on the market, this final analysis of significance remains for some time in the future.

Another complication has arisen in that it has been found that when two inhibitors of cancer in mice (0.05 molar percentage adenosine and 0.08 molar percentage 6 methyl adenosine) are administered into mice from a single solution that a stimulation of cancer growth is obtained.

It is also known that the liver emulsion contains other ingredients than the three nucleosides (Mittelman). There is still the possibility, therefore, that there may be still unknowns which may have an influence on the control of the growth capacity of cancer in mice.

Bearing in mind, therefore, these complications and perhaps others that must be resolved before the final evaluation of the present program on the control of spontaneous tumors in mice may be made the evidence so far obtained justifies the following conclusions.

CONCLUSIONS

1. The sum total of inhibition of tumors obtained with three nucleosides used separately is similar to, if not identical, with the amount of inhibition by the use of the original liver emulsion.

2. There is also a possibility that most of this inhibition of cancerous growth is brought about by 5 methyl cytidine (0.02 molar concentration).

3. Adenosine is not only an inhibitor of cancer in mice but also a stimulator under certain conditions (0.05 molar percentage solution).

4. This conversion of an inhibitor of cancer to a stimulator has been brought about by aging of the solution, which was the means by which the inhibitor in the liver emulsion had been converted to a stimulator of cancer.

5. The mechanism of conversion of an adenosine solution requires further investigation.

REFERENCES

Strong, L. C., and Matsunaga, H. (in press). Differential effects of 3 nucleosides on the growth of spontaneous tumors in mice. Cytobios.