5-Methyl-Cytidine as an Inhibitor of Spontaneous Cancer in Mice

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

Three nucleosides, found in a specially prepared liver emulsion (Mittelman), are inhibitors of the growth of spontaneous tumors of mammary gland origin in mice. The present data demonstrate that 5 methyl cytidine when used in the same molar concentration (0.02) as it is found in the liver emulsion is a powerful inhibitor of spontaneous adenocarcinomata irrespective of age of the cancer proband. More inhibition is obtained in mice between 9 and 12 months of age than between 5 and 8 months. The later appearing tumors (at 9–12 months) grow faster in the controls than those which develop between 5 and 8 months of age. It is reasonable to conclude that 5 methly cytidine has a more inhibitory action on fast growing tumors than upon slower growing ones. However, this inhibitory difference may be mediated somehow or other by a "transmissible entity" or by some other secondary mechanism associated with the aging process. The present series of experiments suggests the possibility that by the simultaneous or tandem use of several nucleosides a broader effect of an inhibitory action on cancer in mice may be forthcoming.

INTRODUCTION

Spontaneous tumors of mammary gland origin in 360 C_3H/ST mice have been used in this experiment. Of these, 304 were used as controls and 56

The authors are from The Leonell C. Strong Research Foundation, Inc., 10457 I Roselle Street, San Diego, California 92121.

Journal of Surgical Oncology

received intraperitioneal injections of a 0.02 molar concentration of 5 methyl cytidine (Sigma) dissolved in distilled water. Due to previous observations that age of onset and size of the tumor and genetic descent of the tumor proband in reference to an injection of a liver extract containing, among other entities a 0.02 molar percentage of a 5 methyl cytidine, are factors in the growth and fate of spontaneous tumors, it became necessary to divide the tumor bearing mice into several classes. The cost of 5 methyl cytidine has handicapped the placement of sufficient numbers to justify a final statistical analysis of the data on the inhibition of tumor growth. Consequently this paper is intended as a preliminary publication of an interesting observation. The final evaluation of tumor growth by 5 methyl cytidine will come when funds are available for the continuation of the present approach to the control of cancer in mice.

It has been ascertained that three nucleosides, when they are injected intraperitoneally into a cancer bearing mouse in the same molar concentration as they are found in a specially prepared liver emulsion, have inhibitory effects on the growth of spontaneous tumors of mammary gland origin. An 0.05 molar solution of adenosine inhibits the growth of spontaneous tumors in 5 to 8 month old mice of the C_3H/ST inbreds but has no, or very little, effect on cancer in a mouse aged 9 to 12 months. An 0.08 molar solution of 6 methyl adenosine also inhibits the growth of spontaneous tumors in 5 to 8 month old mice but not in one aged 9 to 12 months. An 0.02 molar solution of 5 methyl cytidine is, however, a powerful inhibitor of spontaneous tumors in mice irrespective of age of host (Strong and Matsunaga, b).

The purpose of the present paper is to present more data on the suppression of growth rates of spontaneous tumors which has been brought about by an intraperitioneal injection of a 0.02 molar solution of 5 methyl cytidine.

METHODS

The methods of using mice of the well-known inbreds C_3H/ST and C_3HB/ST as a source of spontaneous tumors of mammary gland origin have been reported in a series of papers dealing with the inhibition of such tumors by a liver emulsion and, therefore, need not be repeated in this paper.

The 5 methyl cytidine was placed in a quantity of distilled water so as to form an 0.02 molar concentration preparation. The material was put in a rubber stoppered serum bottle which then was wrapped in aluminum foil in order to avoid exposure to light. When not in use the material was kept under refrigeration, as had the liver emulsion, since the tumor inhibitor or inhibitors were known to be heat sensitive. The 5 methyl cytidine preparation was warmed to room temperature before being injected into mice in order to avoid spasms which usually occur following the injection of refrigerated materials.

RESULTS AND DISCUSSION

The data obtained on the growth rates of spontaneous tumors are presented in Fig. 1. The average growth rate of tumors in control mice between 5 and 8 months of age is on the short dash line. Similar data for control mice between 9 and 12 months of age are on the solid line. The two series of mice receiving periodic injections of 5 methyl cytidine (three per week) are on the two open circle curves. The first series on mice between 5 and 8 months of life receiving 5 methyl cytidine is on the short dash and open circle curve and the second series with mice between 9 and 12 months of age is on the solid and open circle curve.

There is a pronounced inhibition of the growth of spontaneous tumors of mammary gland origin with 5 methyl cytidine in both series of mice based upon the age of onset of the tumor. Thus there has been obtained a verification of the conclusion arrived at previously with the use of 0.02 molar concentration of 5 methyl cytidine (Strong and Matsunaga, a, in press).

The main difference between tumors that arise between 5 and 8 months and those between 9 and 12 months of age is that the tumors developing between 9 and 12 months of age grow faster than those which develop between 5 and 8 months. The architectural arrangements of tumor components in mice of the C_3H/St strain do differ from tumor to tumor and even from area to area of a single tumor, but this varying characteristic is common in mice of either age group. Thus the conclusion may be entertained, for the time being at least, that 5 methyl cytidine appears to affect the growth rate of rapidly developing tumors more than the growth rate of slower growing tumors arising between 5 and 8 months of age.

On the other hand, it has already been demonstrated that both adenosine and 6 methyl adenosine have a significant depressive effect upon slow-growing tumors between 5 and 8 months but apparently little or no effect upon suppressing the growth of rapidly growing tumors between 9 and 12 months of age (Strong and Matsunaga, a).

There is a possibility, therefore, that, by combining two or more of the nucleosides of the original liver emulsion or other nucleosides or even other biochemical compounds in the same solution, a broader effect of the suppression of tumors may be expected. The present data also emphasize the concept that this combination of nucleoside inhibitory materials in the original liver emulsion may also have been a factor in the striking regression of spontaneous tumors by the original liver preparation. As a matter of fact, it has been shown that the sum total of inhibitory effect on tumors by the three nucleosides used separately and in the same molar concentration as they are found in the liver extract is the same as that which was found with the use of the original liver emulsion (Strong and Matsunaga, b, in press).

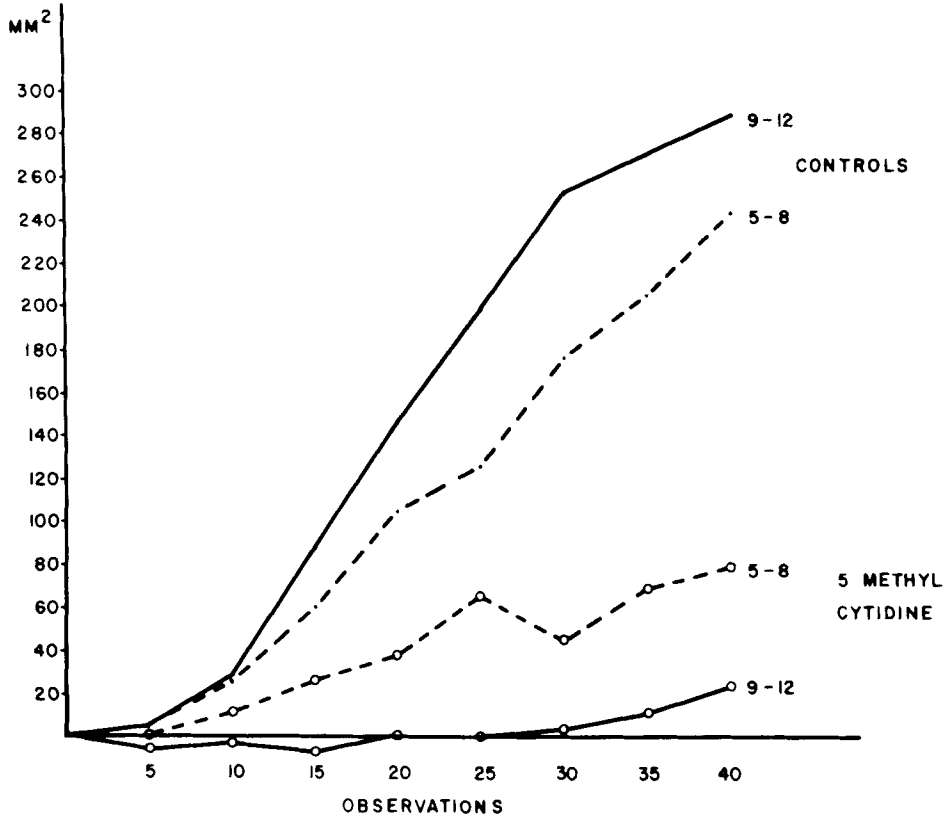

Fig. 1. Average growth rates of tumors are given in mm^2 on the ordinate. Successive tumor sizes spaced at the 5th, 10th, 15th, to 40th periods of observations are on the abscissa.

Bearing in mind, however, that there are many biological differences found in mice of different ages other than the rapidity of growth of spontaneous tumors of mammary gland origin, it may be that another effect of 5 methyl cytidine (or the other nucleosides) upon cancer in mice may eventually be resolved. There is a possibility that the "transmissible entity" discovered in the program of the use of the liver emulsion may be the mediating mechanism for the control of cancer (Strong and Matsunaga, 1970).

ACKNOWLEDGEMENT

This experiment has been made possible, in part, by donations from private individuals, service clubs, labor unions and foundations in the United States

and Canada through the activity of The Friends of the Leonell C. Strong Research Foundation. Recently, Mr. Benjamin Clayton and the Clayton Foundation of Austin, Texas have contributed to the continuation of the research program.

REFERENCES

Strong, L. C., and Matsunaga, H. (in press, a). Comparative effect of three nucleosides on suppression of cancer growth in mice. J. Surgical Oncology. 1972.

Strong, L. C., and Matsunaga, H. (in press, b). Differential effects of 3 nucleosides on the growth of spontaneous tumors in mice. Cytobios 5:119-124-1972.

Strong, L. C., and Matsunaga, H. (1970). A "transmissible entity" in the control of cancer in mice. J. Surgical Oncology 2:363-372.