Journal of Surgical Oncology 7:531–534 (1975)

An Inverse Dose Response of 5-Methyl Cytidine on Multiple Primary Spontaneous Tumors and Their Regressions in Mice

LEONELL C. STRONG, PhD. and HENRY MATSUNAGA, B.S.

Ten series mice of C_3H/ST inbreds with spontaneous tumors have received 5-methyl cytidine (total 500 mice). Graded single doses of (1) 3.86, (2) 2.57, (3) 2.06, (4) 1.54, (5) 1.03, (6) 1.54, (7) 1.03, (8) 0.51, (9) 0.26, and (10) 0.13 mg/injection (three times weekly) were used. In series 1–5 the 5-methyl cytidine was dissolved in distilled water, and in series 6–10, the nucleoside was dissolved in physiological salt solution. In doses administered, series 4–5 overlapped with series 6–7.

The number of mice that showed a regression of tumor increased in the successive series (48%–63% in series 1–5 and 25%–73% in series 6–10). As the number of mice with regressing tumors increased, the percentage of mice showing multiple primary tumors also increased. The administration of the nucleoside in physiological salt solution altered the inhibitory effect of 5-methyl cytidine on spontaneous tumors. For example, in physiological salt solution the 0.51 mg/injection gave as much inhibitory action as 1.03 mg/injection in distilled water. An inverse dose response is thus indicated between the amount of 5-methyl cytidine injected and the inhibition of spontaneous tumors in mice (percentage of regression and number of multiple primary spontaneous tumors are the criteria of inhibition considered).

KEY WORDS: 5-methyl cytidine, regression of tumors, mammary tumors in mice, C_3H

It has been demonstrated that, of the three nucleosides in an alcohol-soluble extract of liver tissue of several species, 5-methyl cytidine is the best inhibitor of the growth of spontaneous tumors of mammary gland origin in C_3H/ST mice (1). It has also been suggested that "there appears to be an active relationship between two inhibitors of cancer." These two inhibitors are 5-methyl cytidine and a "transmissible entity," which has been shown to be transmitted from parent to offspring. "When the effect of the transmissible entity is weak in suppressing cancer, 5-methyl cytidine is a powerful inhibitor of cancer."

The final analysis of the relationship between 5-metyhl cytidine and the transmissible entity will be considered later when both factors have been studied in terms of their effect on the growth and fate of spontaneous tumors.

The purposes of the present paper are (1) to compare the results obtained from mice that showed regression of primary spontaneous tumors with complete disappearance of tumor or eventual recurrence according to the graded doses received and (2) to study the

The Leonell C. Strong Research Foundation, Inc., San Diego, California.
Address reprint requests to Dr. Leonell C. Strong, 10457 I Roselle Street, San Diego, California 92121.

incidence of multiple primary spontaneous tumors appearing in mice of the same graded series.

MATERIALS AND METHODS

Mice of the C_3H/ST and derived C_3HB/ST inbreds were used in this experiment. They were kept as breeders (to brothers) until they developed spontaneous tumors of mammary gland origin. The tumor-bearing mice were then placed in either the control group or received subcutaneous injections of 5-methyl cytidine, the dose varying from 3.86–0.13 mg/injection.

The nucleoside, dissolved in either distilled water or physiological salt solution, was injected three times per week. The tumors were measured by vernier callipers in the two longest diameters. These measurements were multiplied together to indicate the approximate size of the tumor. The analysis of the growing or retrogressive tumor was based upon successive increments – the original size being subtracted from the new size in order to determine the increment or decrement.

The tumor-bearing mice were divided into 10 groups depending upon the dose of the 5-methyl cytidine and the vehicle employed (Table I).

TABLE I. Data on Use of 5-Methyl Cytidine as Cancer Inhibitor in Mice

Series	Vehicle	Route	Mg/Inj	No. Mice	Mg/k
1	DW	Intra-peritoneal	3.86	36	108.0
2	DW	Subcu	2.57	84	71.9
3	DW	Subcu	2.06	40	57.6
4	DW	Subcu	1.54	44	43.2
5	DW	Subcu	1.03	48	28.8
6	PS	Subcu	1.54	20	43.2
7	PS	Subcu	1.03	48	28.8
8	PS	Subcu	0.51	60	19.2
9	PS	Subcu	0.26	60	7.2
10	PS	Subcu	0.13	60	3.6
Controls				312	
Totals				812	

RESULTS

The percentages of mice showing regression of tumor and multiple primary tumor are presented in Fig. 1. It is obvious that as the number of mice regressing their tumors increased (with the reduction in the amount of the nucleoside), the number of mice showing multiple primary tumors also increased.

DISCUSSION

An indirect dose response between the growth rate of a spontaneous tumor of mammary gland origin in a C_3H/ST mouse and the amount of 5-methyl cytidine injected subcutaneously has recently been determined (1). The range of tumor growth rate affected

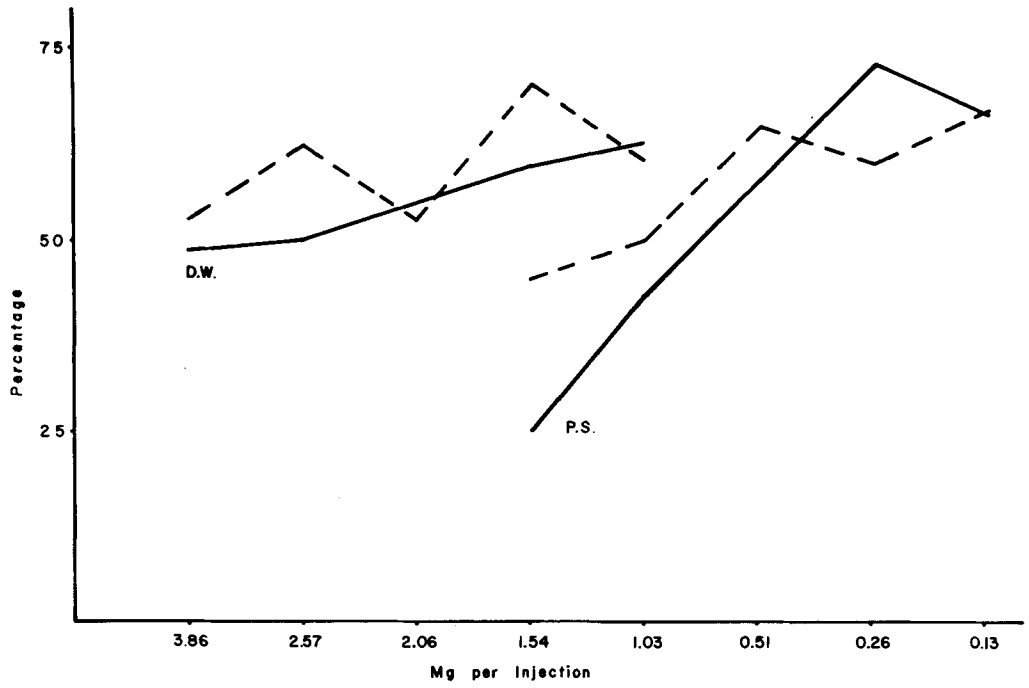

Fig. 1. This figure presents the data on the percentage of regressions of tumors (solid lines) and multiple primary tumors (dash lines). Series 4 and 5 overlap series 6 and 7 having received the same dose of the same nucleoside. Series 4–5 were in distilled water DW and 6–7 were in physiological salt solution. Series of mice are on the abscissae, whereas the percentage of either regressions of tumors or mutliple tumors are on the ordinate.

by 5-methyl cytidine was stimulation with very large doses, to complete stoppage of growth (intermediate doses), and finally regression with an occasional recurrence (small doses). The present data indicate that two other characteristics of malignancy, complete regression and the incidence of multiple primary tumors of mammary gland origin, may also be affected by the administration of 5-methyl cytidine.

It is also evident that the introduction of the 5-methyl cytidine in a solution of physiological salt solution affects the characteristics of malignancy at a different level when compared to solutions of distilled water containing the same amount of nucleoside. It has been demonstrated that 1.03 mg/injection of a physiological salt solution of 5-methyl cytidine will give as much inhibitory effect on cancer as 1.54 mg/injection of a distilled water solution.

The obvious conclusion to be entertained, for the present, is that with a regression a mouse lives longer than one with a growing tumor even though the original primary tumor may have recurred.

However, the C_3H/ST mouse is somewhat unique in that about 80% of them, under controlled conditions, develop multiple tumors. There may be a biological level of malignancy ingrained into a C_3H/ST mouse, and with a loss of the primary malignancy by 5-methyl cytidine, a new tumor arises perhaps in an attempt to maintain this high level of

malignancy. In many studies it has been shown that the C_3H/ST mouse has a higher degree of susceptibility to various kinds of cancer than do mice of any other inbred strain. If so, then it is reasonable to assume that a dose response between 5-methyl cytidine and malignancy may be expected even though this correlation appears to be an inverse dose response relationship.

CONCLUSION

1. 5-methyl cytidine is a more effective inhibitor of cancerous tissue in mice when dissolved in physiological salt solution than when dissolved in a solution in distilled water.
2. The percentage of mice regressing spontaneous tumors of mammary gland origin is increased as the amount of 5-methyl cytidine is decreased (108.0 mg/k of mouse compared gradually to 3.6 mg/k).
3. As the percentage of tumor regression increases, the percentage of primary multiple spontaneous tumors also increases.
4. There may be a biological level of malignancy ingrained in a C_3H/ST mouse.
5. A loss of the primary malignancy by the introduction of 5-methyl cytidine results in an attempt to maintain this high level of malignancy.

ACKNOWLEDGMENTS

This experiment has been made possible by contributions from several foundations, service clubs, labor unions, and private individuals in the U.S.A. and Canada. The Friends of the Strong Foundation, P.O. Box 1130, Escondido, California 92025 have been most helpful in raising funds. Support for the research has also been received from the National Cancer Institute of HEW (grant I ROI Ca 13862-01).

REFERENCES

1. Strong, L.C., and Matsunaga, H. (1973). Comparative effect of three nucleosides on suppression of cancer growth in mice. J. Surg. Oncol. 5:181–188.
2. Strong, L.C., and Matsunaga, H. (1972). Differential effect of three nucleosides on the growth of spontaneous tumors in mice. Cytobios 5:119–124.
3. Strong, L.C., and Matsunaga, H. (1972). Comparison of the effect of three nucleosides and a liver emulsion upon the inhibition of cancer in mice. J. Surg. Oncol. 4:248–254.
4. Strong, L.C., and Matsunaga, H. (1972). 5-methyl cytidine as an inhibitor of spontaneous cancer in mice. J. Surg. Oncol. 4:528–532.
5. Strong, L.C., and Matsunaga, H. (1973). Effect of 5-methyl cytidine on growth and regression of spontaneous tumors in mice. Cytobios 7:253–257.
6. Strong, L.C., and Matsunaga, H. (In Press). Effect of a liver extract and nucleosides on cancer. (Cytobios).
7. Strong, L.C., and Matsunaga, H. (In Press). Further studies on a transmissible entity in relation to the control of cancer in mice. J. Surg. Oncol.
8. Strong, L.C., and Matsunaga, H. (In Press). An inverse correlation (dose-response) between 5-methyl cytidine and the fate of spontaneous tumors in mice. Cytobios.