Further Studies on the Chemotherapy of Adenocarcinoma 755 with 6-Thioguanine*

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SUMMARY

When Ca-755 tumors of various sizes were treated with three doses of C14-labeled 6-thioguanine (10 mg/kg at 24-hour intervals) the specific activities of their deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) at the end of the treatment were independent of the starting size of the tumor. During the subsequent rapid tumor regression the specific activity of DNA and RNA decreased linearly with the amounts present, and when projected the plots cut the specific activity ordinates at values which again were independent of the original tumor size. A second course of thio-
guanine therapy, commenced before tumor recurrence, produced a very much higher proportion of "cures" than did either a single course of therapy or a second course given after tumor recurrence. A second course of therapy with C14-labeled thio-
guanine before recurrence showed that some of the drug was incorporated into the nucleic acid of the remaining tumor cells.

It was recently shown that, when injected on a schedule of three doses of 10 mg/kg at 24-hour intervals, 6-thioguanine (TG) caused an apparently complete regression of established, subcutaneously implanted Sarcoma 180. The same schedule, applied to subcutaneous Adenocarcinoma 755 carried in BDFj mice, resulted in a rapid regression, but this was followed by a recurrence of the tumors. Having recurred, the tumors were less responsive to a second course of drug therapy, and a third course produced little or no response (2).

With the Ca-755 tumors it was also shown that the results obtained were independent of the size of the tumors at the beginning of therapy (over the range 0.7–1.5 cc.). The tumor regression times (2) were closely similar, and the tumors regressed to approximately the same size before recurrence was seen. The purpose of the present paper is twofold. First, to ascertain whether a better response to TG is obtained by treating Ca-755 with a second course of therapy before recurrence; and second, with C14-labeled TG, to further investigate the observation that the response to therapy was independent of the tumor size.

MATERIALS AND METHODS

Ca-755 was inoculated subcutaneously into young adult female BDF1 mice, and the rate of growth and regression and the tumor regression times were measured as previously described (2).

6-Thioguanine (TG) (California Biochemical Corporation) was dissolved in 0.9 per cent NaCl, with the addition of minimal amounts of NaOH, and injected intraperitoneally. Three injections of 10 mg/kg were given at 24-hour intervals. TG labeled with C14 was prepared and donated by Dr. G. A. LePage and was diluted to a specific activity of 0.3 μC/μmole.

The TG incorporation studies were made as follows: groups of mice bearing Ca-755 of varying sizes were treated with C14-labeled TG. Twenty-four hours after the third injection (day 3) four mice from each group were sacrificed, and their tumors were dissected, weighed individually, and homogenized in a rotating pestle glass homogenizer. This procedure was repeated on days 6, 10, and 13. Because of their small size six 13-day tumors were used, and these were pooled before weighing and homogenizing.

DNA and RNA were then extracted from the homogenates and separated by the method of Tyner, Heidelberger, and LePage (9). DNA was estimated by a modified diphenylamine reaction (3) and RNA by the orcinol method (5). Aliquots were also plated and counted by standard procedures in a Nuclear Chicago end-window gas-flow counter (30 per cent efficiency) and corrected for self-absorption.

RESULTS AND DISCUSSION

Specific activities of nucleic acids from Ca-755 during tumor regression.—In separate experiments groups of about 25 mice were given inoculations of Ca-755. The
tumors were allowed to reach a volume of about 0.4, 0.6, 1.0, and 1.4 cc. in each group, respectively, and then eighteen mice were selected with tumors closest to the average size for the group. Each group was then treated with C\textsuperscript{14}-labeled TG, the tumors were harvested at intervals, and DNA and RNA isolated as previously described.

Chart 1 shows plots of total DNA and RNA against their respective specific activities for each tumor group. A linear plot was obtained in all cases. The lines converged and, when projected, cut the specific activity ordinates at closely similar points. This chart also showed that the specific activities were independent of tumor size over the range studied (0.4–1.4 cc. on 1st day of treatment). It was also found (Table 1) that the amounts of DNA and RNA present in the tumor per gm. wet weight remained relatively constant during the regression. Results are not presented after day 10, since the tumors were then very small and necrotic.

Table 1 also shows that the ratio of RNA/DNA in treated tumors was considerably less than that in untreated controls. A similar result was found when S-180 ascites cells were treated with TG or with asaserine + TG (1).

The results in Chart 1 suggest that the average amount of TG taken up into the nucleic acid of each tumor cell was independent of tumor size and that there is an inverse relationship between the survival time of an individual cell and the amount of TG incorporated. The last estimation of specific activity (day 13) is within a few days of the time at which tumor growth normally resumed. This suggests that these cells which incorporated the least TG (the specific activity then averaging about 20 per cent of the value at the end of treatment) were able eventually to resume growth. These data for different tumor sizes agree well with the previously observed (2) results that TG chemotherapy was equally efficient in large or small tumors, and provides additional evidence that the incorporation of the drug into nucleic acid is of primary importance (7, 8).

The convergence on the ordinates of the plots of total DNA and RNA against their respective specific activities (Chart 1) was somewhat unexpected, and there are two possible explanations. First, TG may persist in cells in a sublethal concentration. Second, in a situation where rapid tumor regression occurs after TG incorporation, it seems probable that the necrotic pool will contain nucleic acid breakdown products and that some of the TG content may then be available for re-utilization. The evidence does not so far enable a decision to be made between these alternatives. However, in the case of the 'sub-lethal concentration' alternative, it might be expected that the DNA and RNA specific activity plots would asymptote to the value of specific activity corresponding to such a sublethal concentration, and this situation seems more likely to produce shallow curves than the straight lines observed.

In a further experiment a group of mice bearing Ca-755 (vol., 1 cc. average) was treated with unlabeled TG. On day 10 (day 0 = time of first TG injection) a further course

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Group} & \textbf{Wet wt. (gm.)} & \textbf{DNA (mg.)} & \textbf{RNA (mg.)} & \textbf{RNA/DNA} \\
\hline
\textbf{Group 1:} & & & & \\
Day 3 & 1.3 & 7.4 & 2.8 & 0.38 \\
6 & 0.85 & 5.4 & 2.5 & 0.42 \\
10 & 0.22 & 6.0 & 2.5 & 0.41 \\
\hline
\textbf{Group 2:} & & & & \\
Day 3 & 0.9 & 5.3 & 2.3 & 0.43 \\
6 & 0.37 & 4.4 & 1.8 & 0.41 \\
10 & 0.13 & 5.8 & 2.6 & 0.45 \\
\hline
\textbf{Group 3:} & & & & \\
Day 3 & 0.6 & 5.5 & 2.4 & 0.47 \\
6 & 0.41 & 4.2 & 1.9 & 0.45 \\
10 & 0.14 & 5.5 & 2.0 & 0.39 \\
\hline
\textbf{Group 4:} & & & & \\
Day 3 & 0.37 & 4.8 & 3.0 & 0.62 \\
6 & 0.17 & 4.4 & 2.0 & 0.45 \\
10 & 0.09 & 4.5 & 2.1 & 0.46 \\
\hline
\textbf{Untreated controls} & 0.9 & 7.0 & 4.6 & 0.67 \\
\hline
\end{tabular}
\caption{DNA and RNA Content of Ca-755 During Regression after a Course of Three Injections of Thioguanine (10 mg/kg) at 24-Hr Intervals (Day 0, 1, 2) Compared with Untreated Control Tumors}
\end{table}

Results are given as gm. wet wt. and mg DNA and RNA/gm wet wt. Each figure is the average of four tumors, separately determined, with the exception of the controls, where eight tumors were used.
of injections) showed that, at the total DNA and RNA
less than that obtained with the first course of TG therapy.
second course of therapy resulted in TG incorporation,
activity of the tumor DNA and RNA was estimated. The
i/mole). Twenty-four hours after the third injection with
of therapy was begun with TG labeled with C14(0.3 fie/
four of the remaining seven mice given only one treatment.
slight check, and the remaining four tumors recurred after a
20, 21, 22. In four of these mice tumor growth continued after a
a single course of injection, from day 16 onward (D n),
tumors then continued to grow rapidly, and four of eight
were seen after a single course of TG therapy, but the
(20 per cent) was unusually high. During the previous
experiments (2) a few apparently complete regressions
were seen after a single course of TG therapy, but the
proportion (5–10 per cent) seemed too small to record.
However, in the present experiment 80 per cent of the
tumors recurred with an average regression time of 22
days, which results compare reasonably with the previous
work. These results appear in Chart 2.

The first eight recurring tumors were of a size suffi-
ciently similar to enable them to be treated as a group,
and, when they had reached an average volume of 1 cc.,
they were given a second course of TG therapy. As the
results in Chart 1 show, the growth rate of four of eight
tumors was checked while the TG was being given; the
specific activity of DNA and RNA isolated from the
points marked in Chart 1 left after the first course by the 13th day (when the tumors
points corresponding to 13-day-old tumors, 200–250
counts/min/mg was present in DNA and about 150
counts/min/mg in RNA. In the present experiment
with unlabeled TG used for the first course of injections)
the specific activity of DNA and RNA isolated from the
after the second (10–11–12–day) course of injec-
tions was 195 counts/min/mg and 165 counts/min/mg,
respectively.

Effect of repeated course of TG therapy on the recurrence
of Ca-755.—The results above gave theoretical backing to the
possibility that a second course of TG therapy before
tumor growth recommenced would be effective.

A group of 60 BDF1 mice was given inoculations of
Ca-755. When their tumors had reached a volume of
approximately 0.8 cc. eight were set aside as controls, and
45 were given injections 3 times of 10 mg TG/kg at 24-hour
intervals. The remainder, which had either very large or
very small tumors, were discarded. On day 10, 13, and
15, ten mice were removed and given a further course of
therapy with TG. Ten days is about the shortest inter-
val which can be allowed between courses of therapy to
permit the mice to regain the body weight lost after the
first course. Fifteen days represented the limit of the
time available before recurrence. The fifteen remaining
mice (having received only one course of therapy) behaved
as follows. Eight tumors recurred from about day 17
(regression time, 18 days), and four more from about day
28 (regression time, 29 days). Three mice remained
tumor-free for at least 50 days. This number of “cures”
(20 per cent) was unusually high. During the previous
experiments (2) a few apparently complete regressions
were seen after a single course of TG therapy, but the
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results in Chart 1 show, the growth rate of four of eight
tumors was checked while the TG was being given; the
tumors then continued to grow rapidly, and four of eight
tumors regressed and recurred again after about 10 days.

When the second TG therapy was given before recur-
rence, there were four deaths among 30 mice (from toxic-
ity), five recurrences, and 21 mice were tumor-free at 50
days. On the 54th day these mice were given re-inocula-
tions (other flank) of Ca-755 mice (not shown in Chart 2).
Tumors grew in all mice at the site of this inoculum and
reached an average volume of 1.6 cc. 18 days after inocu-
lation; this showed that the mice were still compatible.
On the 72d day the experiment was terminated, and in no
case was recurrence of the original implant seen. Within
the time period studied (10–15 days after first treatment)
the time at which the second treatment was given made
little difference to the results, except to suggest that at
10 days there was a greater risk of death from toxicity and
at 15 days a greater risk of recurrence.

In a recent paper containing a review of the literature,
Goldacre and Sylvén (4) concluded that the access of a
drug to all the cells of a solid tumor would depend on the
presence or absence of a necrotic center. However, the
present results, and those previously reported with S-180
(1), suggest that TG was in fact able to reach all the cells
in Ca-755 and S-180 tumors, despite the presence of a
fluid and necrotic center, respectively. Nevertheless, the
data of Goldacre and Sylvén (4) do show penetration of
necrotic tumor areas by small quantities of their dyestuff.
It seems, therefore, that viable cells in necrotic areas
are able to incorporate sufficient TG to kill them, although, as
in the case of Ca-755, more than one course of treatment
may be necessary.

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REFERENCES
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