Brief Note

Tritiated Thymidine as a Cytocidal Agent in Human Leukemia

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Cytocidal effects on radiosensitive cells were recognized soon after the preparation of tritiated thymidine (3H-TDR), although no cytotoxic effects had been observed in man from previously employed doses (up to 0.4 μCi/Gm. body weight in a single injection). While giving repetitive injections of 3H-TDR for a study of cell proliferation in a patient with acute lymphocytic leukemia, however, we noted a marked decrease in the number of leukemic cells. This effect could most logically be ascribed to radiation from the 3H-TDR. After similar effects were observed in a second patient, a preliminary communication was felt desirable because of the possible importance of these findings to others studying and managing leukemic patients and because a definitive study would require many years to complete.

Case Reports

Case 1

LU, a 74-year-old woman, entered the Medical Research Center, Brookhaven National Laboratory, September 24, 1964, because of progressive weakness and pallor for two months and nocturnal fever for several days. She had noted scattered, red skin papules for one year. Significant physical findings were a temperature of 38.5 C., pallor, scattered 0.5 cm. red skin papules, coarse rales and expiratory wheezes at the base of the right lung posteriorly, and a spleen which extended 7 cm. below the left costal margin. On admission the hemoglobin was 6.1 Gm. per cent, platelets 18,500/mm.3, and peripheral white blood cell counts (WBC) 43,200/mm.3 with 95 per cent blasts, 3 per cent lymphocytes, and 2 per cent segmented neutrophils. There was an infiltration in the right lower lobe on the chest.

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Research supported by the U. S. Atomic Energy Commission.

Supported in part by USPHS Training Grant #5T4CA-5126-03 from the National Cancer Institute.

First submitted July 19, 1965; accepted for publication May 10, 1966.

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Case 1

PERIOD OF STUDY

Fig. 1.—Case 1 (LU). Total white cells in the peripheral blood per cubic millimeter during entire hospitalization. Both morning and evening blood counts were done during the initial period.

x-ray. A biopsied skin papule was infiltrated by leukemic cells, and a bone marrow aspirate appeared hypercellular with over 90 per cent lymphoblasts. The fever and pneumonia subsided 10 days later after therapy with penicillin, streptomycin, and chloramphenicol. On the fourth day a study of cell proliferation was begun; the patient received 10 intravenous injections of $^3$H-TDR (SpA 1.9 c./mM), each injection 0.25 $\mu$C./Gm. body weight, over a 5-day period. Two weeks later the skin infiltrations had disappeared, and in another week the spleen was palpated only 2 cm. below the left costal margin. A bone marrow aspirate appeared unchanged from the one obtained on admission. One month after the injections, the spleen enlarged again. Two weeks later she developed a right upper lobe pneumonia and died without responding to treatment with antibiotics. Transfusions had been given as necessary. At postmortem examination a right upper lobe lobar pneumonia was found as well as replacement of normal bone marrow and splenic architecture with a uniform population of immature lymphocytes. Serial WBC's are shown in Figure 1. This patient was previously presented, in part, in abstract form.

Case 2

CI, a 42-year-old man who had received the equivalent of 55 rads whole body 80 Kv x-ray in a criticality accident at Los Alamos 18 years previously, developed acute myelocytic leukemia in July, 1964. Methotrexate, 6-mercaptopurine, prednisone, and vincristine given at various times had each produced transitory improvement in the blood counts as well as various toxic manifestations. At the time of referral and admission to the Medical Research Center, Brookhaven National Laboratory, March 30, 1965, the significant physical findings were pallor, extension of the liver 5 cm. and spleen 1/2 cm. below the costal margins, absent deep tendon reflexes, and a mild right foot drop. Initially the hemoglobin was 7.3 Gm. per
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cent, platelets 26,500/mm.\(^3\), and WBC 51,200/mm.\(^3\) with 4 per cent segmented neutrophils, 8 per cent bands, 6 per cent metamyelocytes, 1 per cent myelocytes, 6 per cent promyelocytes, and 75 per cent myeloblasts. A bone marrow aspirate had nearly complete replacement of normal elements by myeloblasts.

Extracorporeal irradiation of the blood (ECIB)\(^\circ\) was begun shortly after admission after the WBC had risen to 209, 000/mm.\(^3\) and he had developed a meningeal leukemic infiltration. ECIB was performed daily during the remainder of his hospitalization. The WBC fell to a "steady state" level of 30–40,000/mm.\(^3\) without apparently affecting the meningeal infiltration. Signs and symptoms of meningeal infiltration disappeared, however, after treatment on April 9 and 20 with intrathecal methotrexate and intramuscular citrovorum factor. The WBC fell to a low of 900/mm.\(^3\) on June 27, then rose rapidly to 69,500/mm.\(^3\) at which time 10 more intravenous injections of \(^3\)H-TDR (SpA 1.9 c./mM), each injection 0.25 \(\mu\)c./Gm., were given every 12 hours (July 5–9). The WBC decreased to 50,000/mm.\(^3\) by the end of this period, then rose immediately to 65,000/mm.\(^3\). After a single intravenous injection of \(^3\)H-TDR (SpA 13 c./mM), 1.0 \(\mu\)c./Gm., on July 13 the WBC again fell to 50,000/mm.\(^3\). After the first series of injections the spleen shrank to the lower costal margin, and the subcutaneous masses decreased considerably in size. A bone marrow aspirate appeared unchanged. The spleen and subcutaneous masses enlarged again shortly after the WBC rose, and their sizes were not affected by the second and third courses of \(^3\)H-TDR. On July 18 he had a grand mal convolution, became comatose, and died. Transfusions had been given as necessary.

Postmortem examination showed massive leukemic infiltrations of bone marrow, thymus, spleen, liver, lymph nodes, parotid glands, kidneys, prostate, testes, epididymis, pleura, dura mater, subarachnoid space, and the subcutaneous, mediastinal, and retroperitoneal soft tissues. Serial WBC's are shown in Figure 2.

Autoradiographs

 Autoradiographs of bone marrow aspirates were prepared with Kodak NTB-2 liquid emulsion and developed after 17 days for Case 1 and 10 days for Case 2. One to two thousand blasts were counted on each slide. Background corrections were made on the basis of slides processed with those above but made prior to the \(^3\)H-TDR injections. The blasts were arbitrarily divided into small (diameter 9–13 \(\mu\)), medium (13–22 \(\mu\)), and large (23–50 \(\mu\)) sizes.

In both patients large and medium blasts, but not small blasts, were proliferating cells with large fractions initially labeled (20–80 per cent). By contrast, less than 5 per cent of the small blasts were initially labeled. Small blasts were apparently derived from the larger ones; this point will be the subject of a separate communication. The per cent of heavily labeled large (see Figures 3 and 4) and medium blasts decreased after the fourth injection of \(^3\)H-TDR in Case 2. Though bone marrow interphase lymphoblasts in Case 1 had as many as 85 grains overlying the nucleus, no mitoses were seen with more than 31 grains (85 and 60 grains, respectively, in Case 2). In Case 2 no specimens were obtained during the second and third courses and only single specimens after each course. The large and medium blasts in Case 2 were only very slightly less heavily labeled after the second course than at the peak of the labeling during the first course and were slightly more heavily labeled after the third.
Fig. 2.—Case 2 (CI). Total white cells, mature white cells, and immature white cells in the peripheral blood per cubic millimeter during the last six weeks of hospitalization. All blood counts were done in the morning.

**DISCUSSION**

Cytotoxic effects of \(^3\)H-TDR have been found in many forms of life. The effects in bacteria and mammalian cells in culture cannot be reasonably compared with man, however, because of the differences in concentrations of, and lengths of exposure to, \(^3\)H-TDR in the medium. Bacterial studies have revealed, however, that the lethal action is due to ionization.7,8 Rupture of the N-ribosyl bond with resultant loss of the pyrimidine base has been suggested as one effect of this ionization.9 Chromosomes manifested single breaks and chromatid exchanges after proliferation in solutions with \(^3\)H-TDR or tritiated water as well as following exposure to \(^{60}\)Co gamma rays.10

Injected \(^3\)H-TDR has produced cytotoxic effects in intact mammals. Mouse11,12 and rat13 spermatocyte numbers were reduced by as little as 1 \(\mu\)c./Gm. body weight in a single injection.11 The proportion of injected \(^3\)H-TDR retained was greater when given in divided than in single doses.12 In the rat a single injection of 25 \(\mu\)c./Gm. produced as much lymphocyte pyknosis as 75 rads of whole-body x-ray, whereas 0.5 \(\mu\)c./Gm. every 12 hours for 17.5 days had no apparent effect.14 Single doses of up to 1 \(\mu\)c./Gm. in the guinea pig, dog, and rat or 0.4 \(\mu\)c./Gm. in man had no apparent effect.5 Early polyploidy was found in the liver of growing rats after 2 \(\mu\)c./Gm.13 Transient deleterious effects on regeneration of the liver in partially hepatectomized rats were noted after 1 \(\mu\)c./Gm.15 In certain tissues having large fractions of cells in DNA synthesis, therefore, toxic effects have been noted with single doses of \(^3\)H-TDR.
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Fig. 3.—Case 1 (LU). Per cent of large blasts in the bone marrow labeled at intervals after the injection of \(^3\)H-TDR. The time scale is expanded for the first 36 hours. Zero time is the time of the first injection of \(^3\)H-TDR.

smaller than the total doses used in the patients reported here. In mice \(^3\)H-TDR has also greatly reduced the survival of normal hematopoietic and lymphoma colony-forming cells. Six injections of \(^3\)H-TDR (SpA 13–19 c./mM), each of which was 47 to 285 \(\mu\)c./Gm., were given at 4-hour intervals. These doses far exceeded those received by our patients. The recent demonstration that leukemic cells have more thymidine kinase than normal cells may mean that these cells can incorporate significantly more thymidine than normal cells during the period of DNA synthesis. Greater incorporation of \(^3\)H-TDR in a given cell would, of course, result in a greater radiation dose rate to that cell.

One can estimate the radiation dose delivered to the nucleus from \(^3\)H-TDR in Case 1 by using the calculations formulated by Bond and Feinendegen. In a cell with an average nuclear diameter of 8 \(\mu\) approximately 7 rads would be delivered in 24 hours if the average activity were 1.2 disintegrations per hour per nucleus. The mean grain count of large bone marrow blasts at one hour was 11.6 after 17 days of autoradiographic exposure, or 0.028 grains per hour. This corresponds to 0.569 disintegrations per hour, assuming an autoradiographic efficiency of 5 per cent. The minimum generation time of these blasts was 21 hours. If the average generation time were 24 hours and the average nuclear diameter were 8 \(\mu\) (not actually measured but a reasonable estimate for these large cells) then the dose during the first generative cycle would be \((0.569/1.2) \times (7)\) or 3.3 rads. Each dividing cell would become labeled again
Fig. 4.—Case 2 (CI). Per cent of large blasts in the bone marrow labeled at intervals after the injection of \( ^3 \text{H}-\text{TDR} \). The time scale is expanded for the first 36 hours. Zero time is the time of the first injection of \( ^3 \text{H}-\text{TDR} \).

every 24 hours if it continued to divide normally—i.e., a total of six times. Each mitosis would halve the intensity of the label. The accumulated dose would then be 3.3 rads at the end of one day, 8.3 rads after two days, 14 rads after three days, 20.2 rads after four days, 26.6 rads after five days, and 33.2 rads after six days. These calculations must overestimate the dose, however, since thymidine is incorporated during the period of DNA synthesis rather than at the beginning of the cycle. On the other hand, those cells which suffered radiation-induced mitotic delay would continue to receive a given dose rate for a longer period of time. To avoid these problems one can state that the average dose rate for a cell labeled as above by a single injection of \( ^3 \text{H}-\text{TDR} \) would be 3.3/24 or 0.14 rads per hour until the first division, 0.07 rads per hour until the second division, etc. With repetitive labeling once every generative cycle, the maximum dose rate would probably converge upon twice the initial dose rate, and increasing numbers of cells would receive large doses. Heavily labeled cells would receive much larger doses than these average figures indicate, and their destruction presumably explains the observed effects. Indeed, the mean grain count of the large blasts which did not enter mitosis was four times as great as that used for the above calculations.

Similar calculations indicate that the average dose rate to the large blasts in Case 2 after a single injection would be 0.15 rads per hour until the first division, etc.

If a tritium disintegration produces 10–20 ionizations within 0.1 \( \mu \) of the
path traversed in a chromosome, and if 15–20 ionizations within 0.1 μ of a chromatin thread are required to produce a chromatid break with a probability of one, then the average large blast in Case 1 would have a chromatid break every two hours until the time of the first division, every four hours until the next division, etc. The chance of such a break being lethal necessarily depends upon the location of the break and upon reparative mechanisms.

The autoradiographic data from the various courses of treatment in Case 2 raise certain questions. The decreases in mean grain count and the heavily labeled segment of large and medium-sized blasts prior to the end of the first course of treatment conceivably could have been caused by the change to ³H-TDR with lower specific activity. This explanation seems unlikely, however, because the second course (consisting entirely of the 1.9 c./mM ³H-TDR) was able to produce grain counts comparable to the peak obtained with the 13 c./mM material and because the results were similar to those in Case 1. The fact that the fall in WBC and decrease in size of leukemic masses after the second and third courses of ³H-TDR were not as great as after the first in spite of comparable grain counts may have been due to (1) destruction of the most radiosensitive cells during the first course of treatment, (2) a greater rate of cell production at the time of the second and third courses, or, in part, (3) destruction of a group of cells exchanging with the blood more rapidly than another group with more limited access to the blood. These questions cannot be answered by our data. Multiple bone marrow specimens during the second course would have answered some of the question but were not obtained unfortunately.

Spontaneous remissions have been noted in acute leukemia, especially in children, after recovery from a severe infection and/or fever. Remissions were often preceded by temporary bone marrow hypoplasia and pancytopenia. Except for the absence of bone marrow hypoplasia and their ages, this description fits our patients. The fall in WBC, decrease in spleen size, and reduction of leukemic infiltrations in both could occur in either a spontaneous or radiation-induced partial remission. In both patients, however, the loss of the heavily labeled segment of proliferating cells in spite of additional injections of ³H-TDR and the apparent inability of the most heavily labeled cells to proceed into mitosis argue strongly for a radiation effect. The differential loss of heavily labeled cells was discernible because of the multiple injections of ³H-TDR; only single injections were generally used in previous kinetic studies.

Although the WBC had fallen after treatment with ECIB in Case 2, the peripheral WBC had oscillated between 30,000 and 50,000/mm³ during the period immediately prior to treatment with ³H-TDR. In addition, the spleen and subcutaneous nodules had been enlarging in spite of daily ECIB. Following the first course of ³H-TDR there was a striking drop in WBC and decrease in size of the spleen and subcutaneous masses. Furthermore, in the experience of Schiffer et al. with ECIB in acute myelocytic leukemia, such a dramatic fall in WBC has not been observed after attaining a new “steady state” level of leukocytes.

The total doses of ³H-TDR used in these patients greatly exceed the maxi-
mum dose (0.4 μc./Gm. in a single injection) previously used in normal or leukemic patients. In these earlier studies no cytotoxic effect was noted.

Whether the clinical observations in these cases are broadly applicable or are unique for two patients with unusually radiosensitive leukemic cells should be further investigated. Certainly our findings suggest caution in interpretation of studies of cellular proliferation derived from ³H-TDR data, and further evidence for cytotoxicity must be sought with the lower doses of ³H-TDR usually used.

**Summary**

1. Two patients with acute leukemia had considerable decreases in leukemic cells in the peripheral blood as well as reduction in size of spleen and leukemic masses after 10 injections of ³H-TDR given over a 5-day period. Each injection was 0.25 μc./Gm. body weight.

2. The pertinent aspects of cytotoxic effects of ³H-TDR are reviewed.

3. The radiation doses delivered to the nucleus are estimated from autoradiographic data.

4. Evidence is presented for the observed effects being due to ³H-TDR.

**Summario in Interlingua**

1. Duo patientes con leucemia acute manifestava declinos considerabile in le numeros del cellulas leucemic in le sanguine peripheric e etiam un reduction in le dimensiones del splen e del massas leucemic post 10 injectiones de thymidina a tritium administrate in le curso de un periodo de 5 dies. Cata-un del injectiones amontava a 0,25 μc per g de peso corporee.

2. Es revistate le aspectos pertinente del effectos cytotoxic de thymidina a tritium.

3. Le doses de radiation recipite per le nucleo es estimate a base de datos autoradiographic.

4. Es presentate evidentia in supporto del these que le effectos observate esseva causate per thymidina a tritium.

**References**


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