Deoxycytidine and Deoxythymidine Kinase Activities in Plasma of Mice and Patients with Neoplastic Disease

Willi Kreis, Zalmen Arlin, Alan Yagoda, Brian R. Leyland-Jones, and Lawrence Fiori

Memorial Sloan-Kettering Cancer Center, New York, New York 10058

ABSTRACT

In C57BL × DBA/2 F₁ (hereafter called BD2F₁) mice inoculated with P815 neoplasms and in AKR mice with spontaneously developing leukemia, significant amounts of plasma deoxycytidine and thymidine kinase activities were detected in advanced disease. Undetectable or low levels of such kinase activities were observed in normal BD2F₁ and in control AKR mice. Initial studies with leukemia patients revealed increased amounts of plasma deoxycytidine and thymidine kinase activities correlating favorably with the peripheral white blood cell counts. Initial studies with small numbers of patients with solid tumors revealed significant activities of both kinases in plasma of patients with four different cancers. Healthy volunteers revealed enzyme activities only insignificantly above background.

INTRODUCTION

One of the goals of an oncologist is the early detection of malignant growth. The evaluation of enzyme activities and enzyme patterns in plasma of cancer patients is well established (6). Such enzymes released into the bloodstream can reflect the metabolic activity of rapidly growing or existing sizable tumors and are routinely used for evaluation of progress and/or response of tumors to treatment.

The present study reports on the evaluation of the activities of 2 scavenger enzymes, dCyd and dThd kinases, in plasma of C57BL × DBA/2 F₁ (hereafter called BD2F₁) mice bearing P815 leukemia, in AKR mice with spontaneous leukemia, and in an initial small number of patients with leukemia or solid tumors. The former enzyme is essential in the initial step of the malignant growth. The evaluation of enzyme activities and/or response of tumors to treatment.

RESULTS

Studies with Neoplasm-bearing Mice. Assays for dCyd and dThd kinase activities in plasma of P815 ascites-bearing mice (Chart 1) (composite curves) over 8 subsequent days reveal an initial small rise from 0.08 and 0.15 nmol/ml/hr. Subsequently, a substantial increase of dThd kinase activity was observed from 0.29 nmol/ml/hr on Day 5 to 21 nmol/ml/hr on Day 7. In comparison, the rise of dCyd kinase activity was delayed by 1 day and was not as prominent as the rise of the activity of dThd kinase (from 0.34 nmol/ml/hr on Day 6 to 3.4 nmol/ml/hr on Day 7). After Day 7, both plasma enzyme activities leveled off. The peripheral WBC increased only slightly over the 8 days of observation, namely, from 6,500/cu mm to 11,500/cu mm, with a transient small decrease from Day 1 to Day 4. The P815 cells, as measured by the total packed cell volume prevailing in the abdominal cavity, increased rapidly from Day 2 to Day 7 and thereafter decreased slightly until Day 8, at which time the mice were moribund and were sacrificed. Normal BD2F₁, mice of comparable age exhibited dCyd and dThd kinase activities of 0.025 ± (S.D.) 0.12 and 0.16 ± 0.65 nmol/ml/hr. An increase of plasma dCyd and dThd kinase activities was also observed in AKR mice with spontaneous leukemia. This increase ranged from undetectable dCyd kinase activity and dThd kinase activity of 0.45 nmol/ml/hr in 5 "normal" mice (with no palpable lymph nodes or spleen) to dCyd kinase and dThd kinase levels of 6.42 and 32.75 nmol/
ml/hr, respectively, in 4 leukemic mice with palpable spleen and lymph nodes.

On blood smears prepared from samples collected from P815 ascites-bearing mice, no P815 cells were detected. In ascites-bearing mice, viability tests of P815 cells performed daily 3 days after inoculation showed cell viabilities ranging from 98% on Day 3 to 91% on Day 7.

Studies with Patients. Evaluations of these kinase activities in 15 healthy volunteers of Memorial Sloan-Kettering Cancer Center with age range of 23 to 57 years revealed levels only insignificantly above background (0.02 ± 0.03 for dCyd and 0.03 ± 0.05 for dThd kinase activities). Sixteen patients with leukemia, 7 of whom had undergone or were undergoing chemotherapy treatment at time of analysis, were evaluated for their plasma dCyd and dThd kinase activities. Table 1 lists initials, age, diagnoses, WBC in increasing numbers, and dCyd and dThd kinase activities. The results indicate a correlation between these kinase activities and peripheral WBC, with correlation coefficients of 0.71 and 0.64 for the 2 activities, respectively. In most cases, the kinase activity levels for the dThd sulfate were moderately to substantially higher than were those for the dCyd substrate. These ratios range from 0.83 to 8.54, with predominance of the range between 2 and 3. In this preliminary series, none of the histological types of leukemias are characterized specifically by low or high dCyd or dThd kinase activities or by specific dCyd:dThd kinase activity ratios.

Twenty-three patients, 12 males and 11 females, with advanced solid tumors were evaluated for plasma dCyd and dThd kinase activities. A large range (Table 2) was observed for the 2 activities, from undetectable levels (levels below the sensitivity of the method) to 26.7 nmol of dCyd and 12.9 nmol of dThd phosphorylated per ml of plasma per hr. Strikingly high plasma levels of both enzymes were observed in the following cases, for dCyd and dThd kinase activities, respectively: fibrohistiocytoma, 10.1 and 12.9 nmol/ml/hr; metastatic prostate carcinoma, 26.7 and 10.6 nmol/ml/hr; metastatic pancreatic carcinoma, 6.7 and 1.2 nmol/ml/hr; carcinoid syndrome, 1.61 and 5.62 nmol/ml/hr. Levels below the sensitivity of the method (0.01 nmol/ml/hr) for these 2 enzyme activities were found in only 5 of the 23 cases studied.

In general, the values for dThd kinase activities were lower than those for the dCyd substrate as reflected in values below 1.0 for the ratios of dThd:dCyd kinase activity. No obvious correlation exists between peripheral WBC and kinase values.

DISCUSSION

The development of increasing dCyd and dThd kinase activity in plasma of P815 ascites-bearing mice is probably correlated to an increasing number of cells accumulating in the abdominal cavity. The correlation, however, is not a direct one;
were released into the culture medium (9). It is likely that the findings of Taylor et al. and our own findings of substantial increase of dCyd and dThd kinase activities in plasma of mice in tissue cultures of hepatoma cells by Taylor et al. (9). The conclusion was substantiated for elevated dThd kinase activity confirmed in studies with Morris hepatoma cells cultured in vitro whereby substantial amounts of dThd kinase activities were increased serum dThd kinase activity was released into the peripheral circulation from tumor cells. These conclusions were applied to human tumors. Increased serum dThd kinase activity was observed in patients with solid tumors with metastases and follow-up of tumor response during radiation treatment of such cancers with 1-ß-D-arabinofuranosylcytosine. Cancer Res., 38, 1105-1112, 1978.

i.e., the sharp increase of ascites cells observed from the first to the fifth day after inoculation precedes the rise of the 2 kinases by 4 and 5 days, respectively. In no instance, even 7 or 8 days after i.p. inoculation of the P815 cells, did we observe any PB15 mast cells in the peripheral blood, confirming the original observation of Dunn and Potter (1). Although a slight increase in the WBC was observed from Day 0 (time of inoculation) to Day 8 (1.8 times), it is unlikely that these increased peripheral WBC are responsible for the sharp rise of the 2 kinase activities, 133 (dThd)- and 44 (dCyd)-fold toward the time of death (Day 8) of the animals. In their study of serum dThd kinase in rats bearing transplanted Morris hepatomas, Taylor et al. (8) came to the conclusion that the significantly increased serum dThd kinase activity was released into the peripheral circulation from tumor cells. These conclusions were confirmed in studies with Morris hepatoma cells cultured *in vitro* whereby substantial amounts of dThd kinase activities were released into the culture medium (9). It is likely that the release of kinases is caused primarily by cell death. This conclusion was substantiated for elevated dThd kinase activity in tissue cultures of hepatoma cells by Taylor et al. (9). The findings of Taylor et al. and our own findings of substantial increase of dCyd and dThd kinase activities in plasma of mice bearing advanced P815 and AKR leukemias indicated the likelihood of increased kinase levels in other neoplasms including those of humans. In many but not all instances, our initial studies in patients with leukemia and solid tumors confirmed our expectations in that respect. In patients with leukemias, with few exceptions, dThd kinase activities in plasma were higher than were the dCyd enzyme activities. The small number of patients does not allow us to make correlations of plasma enzyme levels with the different diagnostic groups of leukemias. Interestingly, the correlation coefficient of WBC versus dCyd kinase activity is more favorable than is the one of WBC versus dThd activity.

In 4 cases, patients with solid tumors exhibited increased amounts of dCyd and dThd kinase activities. The observation of high levels of both activities in patients with carcinoma of the pancreas and a fibrohistiocytoma (up to 336 and 430 times over the mean of normal values) are of particular interest, since biochemical tests for these cancers are highly desirable. The 3 evaluations performed on plasma of patients with prostate carcinomas did not reveal uniform elevations of these 2 enzymes. No obvious reasons were found for the discrepancies observed (1335 and 47 times higher values for dThd kinase activities over those found in plasma of healthy individuals) in 2 patients with prostate carcinoma versus the third one with the same disease and normal kinase activities. Other solid tumors with substantial increases of these enzyme activities in plasma, such as in the rare carcinoid syndrome and others, indicate the need for wider screening of patients with solid tumors. Elevated dThd kinase isozyme activities in peripheral blood lymphocytes and plasma have been described recently in non-Hodgkin's lymphomas (2). High levels of dCyd kinase in solid tumors might indicate a favorable prerequisite for the treatment of such cancers with 1-ß-D-arabinofuranosylcytosine, although this early described tumors in patients with leukemia and solid tumors confirmed our expectations in that respect. In patients with leukemias, with few exceptions, dThd kinase activities in plasma were higher than were the dCyd enzyme activities. The small number of patients does not allow us to make correlations of plasma enzyme levels with the different diagnostic groups of leukemias. Interestingly, the correlation coefficient of WBC versus dCyd kinase activity is more favorable than is the one of WBC versus dThd activity.

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