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Microsomal Enzymes in Patients with Acute Leukemia as Determined by Plasma Half-life of Antipyrine

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Summary. *The metabolism of antipyrine was studied in five patients with acute leukemia, before and after treatment and in relapse, to ascertain the effects of treatment on hepatic microsomal enzyme activity. The mean antipyrine half-life was significantly longer in patients after treatment (15.0 ± 3.7 h) than in patients before treatment (8.8 ± 0.7 h) ($P < 0.02$). Furthermore, the mean antipyrine half-life in patients after treatment was also significantly longer than in patients in relapse (9.2 ± 2.7 h) ($P < 0.05$). Similary, the mean metabolic clearance rate of antipyrine was significantly lower in patients after treatment (26.9 ± 2.2 ml/h/kg) than in patients before treatment (46.7 ± 13.1 ml/h/kg) ($P < 0.02$). The mean apparent volume of distribution was not significantly different during the course of acute leukemia. The mean transaminase activity in patients after treatment was significantly higher than that in patients before treatment and in relapse. Thus, treatment with antileukemic agents and blood transfusions might alter hepatic microsomal enzyme activity.*

Introduction

As liver is a principal site of drug transformation and is assumed to be capable of influencing the disposition of many drugs, patients with liver disease might be expected to show a defect in the elimination of drugs that are metabolized by the liver [5]. There are many possible etiologies of liver disease occurring during the course of acute leukemia. They include viral hepatitis, hepatitis associated with sepsis, leukemic infiltration, hepatotoxic effects of antileukemic agents, and concurrent primary hepatic disease [3]. The complicating liver disease may lead to modifications of the doses of antileukemic agents or even the deletion of potentially useful agents, since patients usually receive a number of antileukemic agents which are metabolized by hepatic microsomal enzyme [8, 14].

We, therefore, investigated the possibility that treatment of leukemia with antileukemic agents and blood transfusions might alter drug metabolism, using antipyrine half-life as an index of drug metabolism to evaluate the microsomal enzyme system in humans with acute leukemia.

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Materials and Methods

Serial antipyrine metabolism was studied in 15 instances (5 patients with acute leukemia). The patients included in this study had the following types of leukemia: AML (3) ALL (1), and erythroleukemia (1), five being men and one a woman. The age of the patients ranged from 16 to 65 years. Antipyrine metabolism was studied in all of them before and after treatment and during relapse. Five in-patients with peptic ulcer acted as control subjects as described previously [13]. Treatment was with DCMP combinations (daunorubicin, cytosine arabinoside, 6-mercaptopurine and prednisolone) and blood transfusions, according to the treatment protocol shown below.

Daunorubicin: 20 mg day 1 only, IV
Cytosine arabinoside: 120 mg/day × 14 days, IV
6-Mercaptopurine: 120 mg/day × 14 days, PO
Prednisolone: 30 mg/day × 14 days, PO

Courses were repeated every week until severe leukopenia (wbc less than 1,200/mm) occurred. Four of the five patients received two courses and one received one course. Complete blood counts were obtained at least three times a week. Serum and urine creatinine, serum albumin, total bilirubin, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were monitored weekly, or more frequently if renal or hepatic dysfunction was encountered. Antipyrine metabolism was studied 14–21 days after the last dose of cytosine arabinoside, 6-mercaptopurine and prednisolone. When remission was attained, no patients were placed on maintenance therapy. The mean duration of remission was 4.5 months, lasting 3–6 months in five patients. Antipyrine metabolism was also studied before reinduction.

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Table 1. Biochemical summary in five patients with acute leukemia

	Creatinine clearance (ml/min)	Serum albumin (g/dl)	Total bilirubin (mg/dl)	GOT (mU/ml)	GPT (mU/ml)
Pretreatment	118.8 ± 31.3[a]	4.1 ± 0.3	0.46 ± 0.17	25 ± 14	41 ± 27
After treatment	121.2 ± 26.9	3.7 ± 0.5	0.90 ± 0.44	105 ± 45[b]	171 ± 30[c, d]
Relapse	109.8 ± 22.2	4.4 ± 0.4	0.55 ± 0.19	57 ± 25	82 ± 47

[a] Mean ± SD
[b] Significantly different from before treatment ($P < 0.01$)
[c] Significantly different from before treatment ($P < 0.001$)
[d] Significantly different from in relapse ($P < 0.02$)

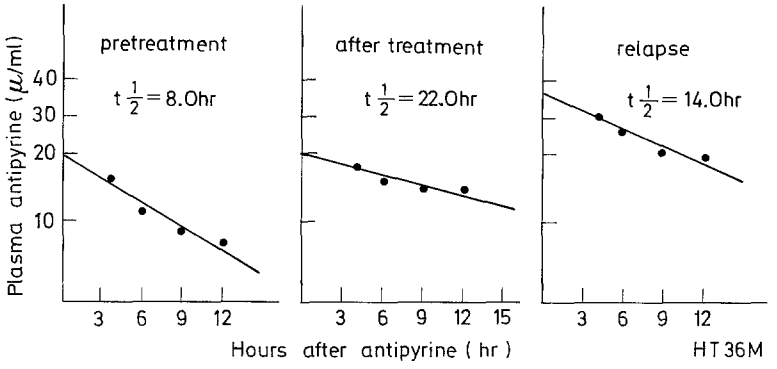

Fig. 1. Decline of antipyrine in the plasma of a patient with AML

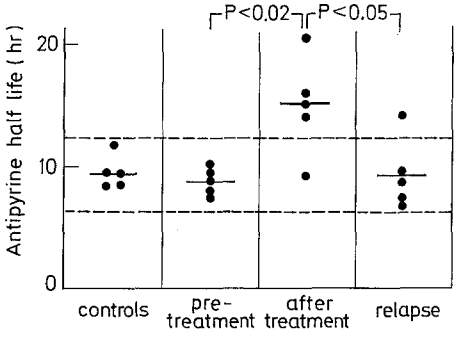

Fig. 2. Antipyrine half-life in five patients with acute leukemia

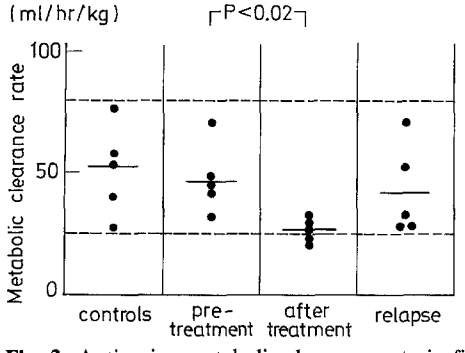

Fig. 3. Antipyrine metabolic clearance rate in five patients with acute leukemia

After overnight fasting, antipyrine (1.0 g) was given by mouth with 300 ml water. No food was permitted for 3 h thereafter, to ensure complete absorption of the drug. Blood was drawn into tubes containing sodium heparin, 4, 6, 9, and 12 h after drug administration. The plasma antipyrine level was determined by the method of Brodie et al. [6]. Log concentration vs time was plotted, and the elimination rate constant (Ke), plasma concentration at zero time (Co), and antipyrine half-life ($t_{1/2}$) were calculated by the methods of least squares. The apparent volume of distribution (aVd) and metabolic clearance rate (MCR) were determined from the following equations:

$t_{1/2} = 0.693/Ke$ (1)
$aVd = dose/Co$ (2)
$MCR = aVd.Ke$ (3)

None of the patients was receiving chemotherapy at the time of this study.

Results

Table 1 shows the mean values for the biochemical parameters, which reflect the presence of hepatic disease before and after treatment, and in relapse. The mean creatinine clearance was similar. The serum albumin concentration after treatment was lower than that before treatment and in relpase, but they were not significantly different. Total bilirubin after treatment was higher than that before treatment and in relapse, but they were also not significantly

Table 2. Pharmacokinetic data on antipyrine in five patients with acute leukemia

	Co (μg/ml)	aVd (l/kg)	MCR (ml/h/kg)	$t_{1/2}$ (h)
Pretreatment	37.0 ± 10.2[a]	0.586 ± 0.122	46.7 ± 13.1	8.8 ± 0.7
After Treatment	30.6 ± 5.9	0.580 ± 0.177	26.9 ± 2.2[b]	15.0 ± 3.7[b,c]
Relapse	40.2 ± 6.0	0.483 ± 0.174	42.4 ± 17.1	9.2 ± 2.7

[a] Mean ± SD
[b] Significantly different from before treatment ($P < 0.02$)
[c] Significantly different from relapse ($P < 0.05$)

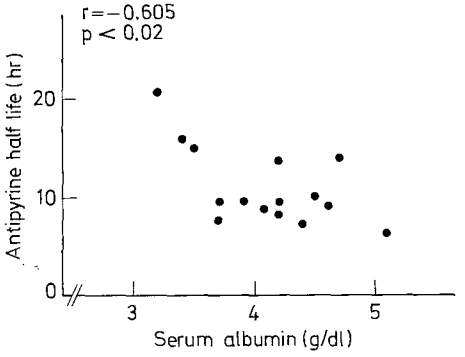

Fig. 4. Antipyrine half-life and albumin in patients with acute leukemia

different. GOT and GPT after treatment were significantly higher than those before treatment and in relapse ($P < 0.01$, $P < 0.001$). GPT after treatment was also significantly higher than that in relapse ($P < 0.02$). In each case, the GOT and GPT levels were normal or only slightly elevated at the time of diagnosis of acute leukemia. The enzyme levels and total bilirubin tended to increase with treatment, and then returned toward normal before the next treatment. In all instances, the values in Table 1 were obtained on the days of administration of antipyrine. The serum for all subjects was negative for hepatitis B antigen. All patients received blood transfusions in their initial hospital course.

Pharmacokinetic data are shown in Table 2 and in Figs. 1, 2, and 3. The concentration of antipyrine in plasma was plotted as shown in Fig. 1, which illustrates the rate of drug elimination from plasma for a patient with AML before treatment, after treatment, and in relapse. The half-life calculated from these regression equations in a patient with AML indicated that the half-life was 8.0 h before treatment, 20.0 h after treatment, and 14.0 h in relapse. As shown in Table 2, the mean half-life after treatment was significantly longer than that before treatment and in relapse ($P < 0.02$, $P < 0.05$). All the patients before treatment show normal half-life when normal range is expressed as mean ± 2 SD of control subjects (Fig. 2). Similary, the mean metabolic clearance rate after was significantly lower than that before treatment ($P < 0.02$). All of the patients before treatment and in relapse show normal metabolic clearance rates when the normal range is expressed as mean ± 2 SD of control subjects (Fig. 3). The treatment of acute leukemia with antileukemic agents and blood transfusions was followed by a decrease in the rate of disappearance of antipyrine from plasma. The rate of disappearance of antipyrine was increased in relapse with recovery of liver function. The mean plasma concentration at zero time and the mean apparent volume of distribution were not significantly different during the course of acute leukemia.

Some reports have suggested that malnutrition and impaired liver function may lead to decreased levels in the activity of hepatic microsomal enzyme in humans [1, 5]. The relationship between the half-life of antipyrine and albumin concentrations in patients with acute leukemia is studied in Fig. 4. A significant negative correlation was observed between the half-life and albumin concentrations in patients with acute leukemia ($n = 15$, $r = -0.605$, $P < 0.02$). It appears that the lower levels of serum albumin were observed in patients who exhibited longer half-lives.

Discussion

The present study indicates that abnormal antipyrine metabolism may occur in patients receiving chemotherapy and blood transfusions. The alterations in antipyrine metabolism seen in this study were associated with alterations in the results of the traditional liver function test. Antipyrine is widely used to evaluate the hepatic microsomal enzyme system, since it is rapidly and almost completely absorbed from the gastrointestinal tract, is evenly distributed in the total body water, and is metabo-

lized slowly, and only the major metabolite-4 hydroxy-antipyrine is excreated in urine [17]. Thus, data from these experiments, strongly suggest that treatment of acute leukemia with antileukemic agents and blood transfusions might diminish hepatic microsomal enzyme activity.

The possible etiologies of liver disease occurring during the course of acute leukemia are many and varied. They include viral hepatitis, hepatitis associated with sepsis, leukemic infiltration, drug effects, and concurrent primary hepatic disease [3]. In each case, the GOT and GPT levels were normal or only slightly elevated at the time of diagnosis of acute leukemia. Five patients had the liver function abnormalities at the time of hematologic remission. Furthermore, the enzyme level declined in relapse. We are not, therefore, aware of any instances of clinically significant leukemia liver infiltration in acute leukemia. Two possible correlations were suggested. All patients received blood transfusions in their initial hospital course. Thus, the possibility of a transfusion-related hepatitis cannot be excluded. The other more striking correlation related to the administration of induction chemotherapy. All five patients had previously received drug combinations previously implicated in causing abnormalities in liver function, such as 6-mercaptopurine or cytosine arabinoside [4, 9].

The results of this study suggest that the cause of the prolongation of the half-life of antipyrine in patients with liver disease might be defective synthesis of microsomal enzyme protein. Firstly, decreased activities of hepatic microsomal enzyme have been reported in rats treated with some anticancer agents [7]. It may be predicted that these substances, such as antimetabolic and alkylating agents, which interfere with the synthesis of nucleic acids, would inhibit hepatic microsomal enzyme. Secondly, the impairment of antipyrine elimination is greater when hepatocellular function is compromised. A low serum albumin is well established as a feature of failing function of the liver parenchyma, representing a failure of protein synthesis. The lower levels of serum albumin concentrations were obtained in patients who exhibited longer antipyrine half-life.

The effect of the presence of some tumors on the hepatic microsomal enzymes that metabolize drugs have been studied extensively in animals. These studies show that inhibition of hepatic drug metabolism is observed in tumor-bearing animals [15, 16]. There was a good correlation between the tumor size and enzyme inhibition. Furthermore, injection of tumor extracts to normal rats produced an inhibitory effect. These results indicate that humoral factor from neoplasia may inhibit drug disposition and metabolism in animals. In contrast with animal studies, little is known about drug metabolism in humans with cancer. Ambre et al. [2] reported a shortened plasma elimination half-life of antipyrine in patients with lung cancer than in normal volunteers. In contrast with the findings of Ambre et al. [2], Tschanz et al. [18] recently demonstrated a decreased rate of drug clearance in lung cancer patients. All the patients before treatment show normal half-life, and the mean half-life in patients at time of hematologic remission after treatment was significantly longer than that in patients before treatment. These observations indicate that the changes in half-life of antipyrine might not be primarily due to the presence of tumor.

Because of the effects of treatment with antileukemic agents and blood transfusions on drug elimination observed in our studies, the effects of treatment on the disposition of antileukemic agents used to treat patients with leukemia should be carefully examined. Cyclophosphamide requires biotransformation into alkylating derivatives in order to exert its pharmacologic effects. This biotransformation is mediated by hepatic microsomal enzymes [8]. On the other hand, nitrosourea is inactivated by hepatic microsomal enzymes [14]. These results suggest the possibility that treatment of leukemia with antileukemic agents and blood transfusions could either increase or dicrease the effects of drugs in man. Anticancer agents and extracts from bacteria are also currently used in combination for the immunotherapy of a number of neoplastic diseases. Recent studies suggested that extracts from bacteria cause a significant reduction in hepatic microsomal activities [10–12]. Because of these observations, information about changes in drug metabolism in patients with acute leukemia deserves serious consideration.

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