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Clinical and Pharmacological Studies with 5-Hydroxy-2-formylpyridine Thiosemicarbazone[1]

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SUMMARY

5-Hydroxy-2-formylpyridine thiosemicarbazone (5-HP) is the first of a relatively new class of antineoplastic agents, the α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, to be studied in man. This study characterizes the toxicity and pharmacological disposition of the drug in 13 patients. Plasma levels of 5-HP decayed in biphasic mode with an initial half-life of 2.5 to 10.5 min. Of the administered dose, 47 to 75% was excreted within 24 hr, and the major metabolites (50 to 74% or urinary radioactivity) were glucuronide conjugates. A characteristically dark green urine resulted from the excretion of significant amounts of iron (2 to 11 mg/24 hr) in chelate form with 5-HP. The incorporation of thymidine-^{3}H into DNA was inhibited in isolated normal and leukemic leukocyte suspensions after exposure *in vitro* and *in vivo* to 5-HP. Transient decreases in blast counts were observed in three of five patients with acute leukemia, although no remissions were obtained. No antitumor effects were noted in eight patients with solid tumors. Administration of larger doses of drug was limited by gastrointestinal toxicity. Mild myelosuppressant effects and hemolysis were noted in five patients treated with 5-day courses of drug. While toxicity appears to limit the usefulness of this compound as an antineoplastic agent, antileukemic activity was shown, and studies of other members of this class of agents are warranted.

INTRODUCTION

Many α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones have shown significant antitumor activity against experimental neoplasms. These compounds are typically excellent coordinating agents for transition elements such as iron, cobalt, nickel, copper, zinc, and manganese (12, 14). This property enables such compounds to chelate the iron, which is a cofactor in the ribonucleoside diphosphate reductase enzyme system, thus limiting the availability of deoxynucleotides and thereby inhibiting the synthesis of DNA (1, 4, 15, 16). Such inhibition apparently serves as the basis of their carcinostatic and antiviral activities (4, 5).

The first of these compounds to have reached clinical trial is 5-HP[3] (Chart 1), a derivative that showed considerable activity in preclinical studies (3, 7, 13) and which has a sodium salt, the water solubility of which facilitates parenteral administration. By contrast, IQ-1 (Chart 1) is a more potent inhibitor of the reductase enzyme (2, 16), but is insoluble in aqueous media.

This study describes our Phase I experience with 5-HP. Reports of preliminary clinical and pharmacological data have been presented (10, 11).

MATERIALS AND METHODS

Patient Selection and Evaluation

Thirteen patients were treated. Three patients with acute lymphatic leukemia and 2 patients with myelomonocytic leukemia had relapsed after multiple courses of drug therapy. Six patients with advanced colon cancer metastatic to liver were included in the study; all had become resistant to 5-fluorouracil, and 4 had received 1,3-bis(2-chloroethyl)-1-nitrosourea. One patient with advanced malignant melanoma resistant to 1,3-bis(2-chloroethyl)-1-nitrosourea and vincristine and another with metastatic breast carcinoma resistant to methotrexate, cytosine arabinoside, cyclophosphamide, and 5-fluorouracil were also treated.

Patients were hospitalized for study and treatment in the Clinical Pharmacology and Oncology Research Unit of the Yale-New Haven Medical Center, and written informed consent was obtained. Pretreatment assessments of the extent of disease were made by physical examination, X-ray, peripheral blood and bone marrow findings, and multiple studies of liver and renal function. Patients were carefully observed for symptoms and signs of drug toxicity as well as for antineoplastic effects. Serial hematological, hepatic, and renal function studies were performed as indicated. Twenty-four-hr urine collections were obtained prior to, during, and immediately after the periods of drug treatment.

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[3] The abbreviations used are: 5-HP, 5-hydroxy-2-formylpyridine thiosemicarbazone; IQ-1, 1-formylisoquinoline thiosemicarbazone; PCA, perchloric acid; TCA, trichloroacetic acid.

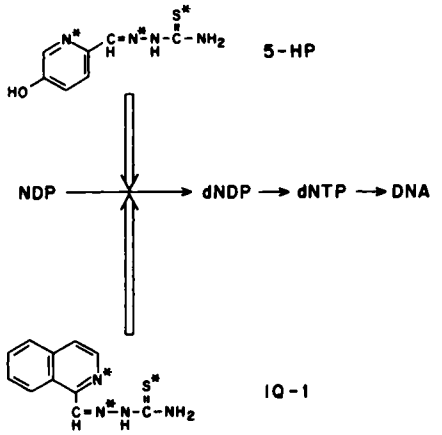

Chart 1. Structural formulas and site of action of 5-HP and IQ-1; the N*–N*–S* coordinating function is indicated. *NDP*, *dNDP*, and *dNTP* on the metabolic pathway represent purine or pyrimidine ribonucleoside diphosphates and the corresponding deoxyribonucleoside diphosphates and triphosphates, respectively.

Determinations of urinary calcium, phosphate, lead, copper, and zinc were done according to standard laboratory methods. Iron was measured by atomic absorption by a modification of the method of Olson and Hamlin (18).

Chemicals

5-HP was provided by Dr. Steven Carter of the Cancer Therapy Evaluation Branch of the National Cancer Institute. Thymidine-methyl-^{3}H was purchased from Tracerlab, Inc., Boston, Mass., and elemental ^{35}S was purchased from New England Nuclear, Boston.

Preparation of Labeled 5-HP

Labeling of 5-HP with 35 S, as with IO-1 (6), was accomplished by adding 2 mCi of elemental ^{35}S dissolved in 1 ml of benzene to a solution of 98 mg of 5-HP in 0.5 ml of dimethylformamide and heating the solution at 125–130° for 2 hr in an oil bath, allowing the benzene to distill off. Then 10 ml of H_2O and 50 mg of Norit were added, and the mixture was heated to boiling, filtered, and allowed to crystallize at 4°. The product gave only 1 spot migrating with 5-HP when subjected to thin-layer chromatography (*n*-butyl alcohol saturated with H_2O) and represented a 90% exchange of sulfur in 5-HP with ^{35}S, for a yield of 70 mg.

5-HP labeled at position 3′ of the side chain was synthesized by reacting 5-hydroxy-2-formylpyridine with ^{14}C-labeled thiosemicarbazide prepared as described previously (6).

Administration of 5-HP

Drug in the form of the sodium salt was administered i.v. either by a Volutrole, which delivered single doses of 1 to 16 mg/kg in approximately 6 min, or by slow, continuous i.v. infusion. Initially, 1 mg/kg was given as a single dose. Both the single dose and the number of injections per day were gradually increased; the largest single dose given was 16 mg/kg. One patient received as many as 8 injections in a day, although most received drug 4 times/day at 6-hr intervals. The intervals between days of drug therapy were gradually decreased, and 5 patients were treated with 5-day courses of 4 mg/kg every 6 hr by injection. Drug was also administered by continuous infusion in 5-day courses to 3 patients at doses of 8, 16, and 32 mg/kg/day.

Incorporation Studies

Leukocytes. Preparations of normal and leukemic human leukocytes were obtained by sedimentation of heparinized blood with an equal volume of 6% dextran in 0.9% NaCl solution followed by exposure of the cells to distilled water for 20 sec to lyse contaminating red cells (9). Final cell suspensions were made in Eagle's medium or Krebs-Ringer phosphate buffer at pH 7.4, supplemented with 10% fetal calf serum. 5-HP or IQ-1 was added to the suspension at concentrations between 10^{-6} and 10^{-4} M. After a 15-min preincubation, thymidine-methyl-^{3}H (2 μCi; 3.0 Ci/mmole) was added, and the samples (final volume, 4 ml) were incubated for 30 min at 37°. Reactions were terminated by the addition of an equal volume of PCA (1.0 M), and the precipitates were washed repeatedly with cold 0.5 M PCA to remove acid-soluble radioactivity. The residues were finally heated at 85° for 30 min with 2 ml of PCA (0.5 M), and the radioactivity in the PCA extracts was measured with a Packard Tri-Carb liquid scintillation spectrometer.

Whole Blood or Bone Marrow. Blood or marrow samples (1 ml) containing 40 units of heparin per ml were added to Krebs-Ringer phosphate containing 5-HP or IQ-1, and the flasks were preincubated for 15 min before the addition of labeled thymidine or uridine as described for leukocytes to give a final volume of 4 ml. Samples (1.5 ml) were removed at 0 and 30 min and added to 8 ml of cold TCA (10%, w/v); this acid gives more satisfactory precipitation of whole blood than does PCA. The residues were washed thoroughly with cold TCA and then hydrolyzed at 85° for 30 min with 3 ml TCA. The acid supernatants were assayed for radioactivity.

Studies of the Metabolism of 5-HP

Patients received 5-HP, 1 to 16 mg/kg, containing ^{14}C (position 3′) or ^{35}S at levels of 42.6 to 96.0 X 10^6 dpm, by either rapid administration or 6-hr infusion. Serial blood and urine samples were obtained, and plasma was separated from heparinized whole blood by centrifugation. Radioactivity in urine, plasma, and cerebrospinal fluid (lumbar puncture) was determined by liquid scintillation spectrometry. Plasma and whole blood samples (0.2 ml) were also plated onto stainless steel planchets with 8 drops of 0.2 N NaOH solution, dried at room temperature, and counted in a windowless Nuclear Chicago gas-flow counter. Radioactivity in expired air was measured by having patients exhale through either a saturated aqueous solution of barium hydroxide to trap $^{14}CO_2$ as barium carbonate or 95% ethanol to trap any volatile ^{35}S label. Barium carbonate precipitates were dried and reduced to a fine powder, and 100-mg samples were weighed and their radioactivity measured by liquid scintillation techniques.

Standardization was accomplished by counting in a similar fashion barium carbonate precipitated from a known solution of sodium bicarbonate-^{14}C by adding barium chloride.

Processing of Blood and Urinary Samples.

Plasma was deproteinized with PCA (10%) and neutralized with KOH before the samples could be subjected to chromatography. The supernatants were lyophilized and reconstituted before application to thin-layer plates. In some cases, both deproteinized plasma and urine samples were extracted at pH 7 with water-saturated ethyl acetate, and the extracts were chromatographed; this extraction procedure is selective for 5-HP (22). Urine samples were also incubated for 2 to 18 hr with β-glucuronidase (0.03 Worthington units/ml) before and after chromatographic separation to determine the amount of glucuronides present. Free glucuronic acid, liberated after hydrolysis, was detected with naphthoresorcinol. Attempts were made to detect ethereal sulfates by a method involving concentration and acidification of acetone-soluble urine fractions (20).

Fractionation of urinary radioactivity on columns of AG-1-X8 (Bio-Rad Laboratories, Richmond, Calif.) anion-exchange resin, formate form, was carried out in a fashion similar to that previously described (8), but with a modified elution scheme. Urine samples were applied to the columns, which were then eluted with 50 ml of H_2O, 120 ml of 0.1 N formic acid (this eluted 5-HP), and at least 200 ml of 2 M ammonium formate (eluted glucuronides and inorganic sulfate); fractions were collected in 10-ml aliquots. Thin-layer chromatography was performed with 0.5-mm layers of Silica Gel H (Merck & Co., Inc.) on 20- x 20-cm glass plates in *n*-butyl alcohol : formic acid : H_2O (77:10:13, v/v). R_F values were as follows: IQ-1, 0.72; 5-HP, 0.82; 5-HP glucuronide, 0.26; urea, 0.54; thiourea, 0.75; semicarbazide, 0.14; and thiosemicarbazide, 0.57. For descending paper chromatography on Whatman No. 3 paper with the same solvent system, R_F values were: urea, 0.37; thiourea, 0.36; semicarbazide, 0.07; and thiosemicarbazide, 0.18. This last group of compounds was visualized by spraying the chromatogram with 4-dimethylaminobenzaldehyde. These latter compounds could be formed by catabolism of the side chain of 5-HP and would elute from anion exchange columns in the H_2O wash. A few plasma samples were subjected to gel filtration on 20- x 1-cm columns of Sephadex G-25; these columns were eluted with 0.005 M NaCl to separate drug-derived radioactivity associated with plasma protein from free, low-molecular-weight compounds.

RESULTS

Acute Effects of Drug Administration. Symptomatic toxicity became evident within 15 to 60 min after a single dose of 5-HP, 4 mg/kg; mild to moderate nausea and occasional vomiting developed in 5 of 9 patients. At 8 mg/kg, the gastrointestinal effects became more severe; of 4 patients, 2 developed profuse vomiting, diarrhea, and fever (>101°). Sixteen mg/kg were administered to only 2 patients, but this dose intensified toxic manifestations, with 1 patient developing tachycardia and transient hypotension.

Administration of drug by constant i.v. infusion appeared to ameliorate these symptoms. Doses of 8, 16, and 32 mg/kg/day were given without the acute time-related onset of nausea and vomiting; less severe and intermittent nausea with occasional vomiting was observed.

Hematological Effects of 5-HP Therapy. The hematological effects of 5-HP were best characterized in 5 patients with solid tumors not involving the bone marrow, who were treated by injection of 5-HP, 4 mg/kg, every 6 hr for 5 days. Comparison of posttreatment marrow aspirates with pretreatment samples revealed modest megaloblastic changes in 2, but hypocellularity was not documented. Two patients developed modest leukopenia, with nadir counts of 2,900/cu mm occurring on the 9th and 10th day after therapy was initiated. Two patients had platelet counts of 17,000/cu mm and 36,000/cu mm at their nadir. Decreases in hematocrit of >5% occurred in 4 of the 5 patients. Mild elevation of bilirubin during therapy was evident in all 5 patients; peak values above 1 mg/100 ml were found in 2 patients who developed 18 and 42% nucleated red blood cells in the peripheral blood. One of these patients had a marked reticulocytosis (14%) and absent serum haptoglobin, confirming a hemolytic reaction. The hematocrit decreased from 41 to 27% but returned to normal without transfusion after therapy was discontinued. The effects of drug therapy on normal hematopoietic elements in patients with leukemia could not be accurately assessed. Preexisting anemia, granulocytopenia, and thrombocytopenia were present in all patients, and all patients received red blood cell and platelet transfusions during the treatment period or immediately afterwards.

Metal Chelation by 5-HP. The initial urine samples collected after 5-HP administration varied from dark green to intense brown after larger doses of drug. Color intensity appeared to correlate with marked increases in the excretion of iron at all dose levels; serum iron and total iron-binding capacity of the serum also increased. Urinary iron excretion ranged from 2.0 to 11.0 mg in 24 hr, compared to control values of 0.1 to 0.3 mg. The maximum excretion of 11.0 mg in 24 hr occurred in a patient with acute leukemia. Iron excretion during 5 consecutive days of therapy remained relatively constant. The average daily excretion ranged from 4.5 to 6.5 mg during the treatment period and returned to normal promptly after the drug was discontinued. There was no change in the amount of unstained iron visualized in posttreatment bone marrow aspirates. Urinary and serum calcium, copper, and zinc values remained within normal limits during treatment. Increased phosphaturia (>1 g/24 hr) was noted in 5 patients during the 24-hr period immediately following drug treatment, although serum phosphate levels were unchanged.

Metabolism of 5-HP. Plasma levels of radioactivity decayed with half-lives of 15 to 60 min after rapid administration; after i.v. infusion the maximum level was achieved at the end of the infusion. In 1 subject (Case 3) who received 5-HP in both isotopic forms, the curves for clearance of ^{14}C and ^{35}S label were identical. When they were determined, levels of radioactivity in the cerebrospinal fluid, as percentage of the coincident plasma levels, varied from 0.8 in a 1-hr sample to

48 in a 4-hr sample but mostly were close to 20. The amount of radioactivity present as unchanged 5-HP was determined by chromatographic separation of neutralized PCA extracts of plasma. It was observed that the plasma levels of 5-HP decreased in biphasic fashion after rapid i.v. administration. The maximum plasma concentrations of 5-HP and the half-lives ($t_{1/2}$) of the initial and secondary clearance curves are given in Table 1. The initial rapid phase was not seen for the subject given a slow infusion of 5-HP, as would be expected if this phase reflects the contribution of absorption and distribution in tissues in addition to excretion. During the 3rd course (September 11) of treatment in Case 2, the initial $t_{1/2}$ could not be determined with the number of points available, but it does appear that initial clearance at that time was rapid, since maximum levels of 5-HP in plasma were lower.

In Case 1, plasma samples taken during the 1st 3 hr were subjected to gel filtration on Sephadex G-25. Results indicated that 29 to 34% were associated with plasma protein. Similar association was demonstrated *in vitro* by adding 5-HP-^{14}C to unlabeled plasma from the same patient and separating the protein-bound fraction with Sephadex.

In 2 patients, 70% of the total administered dose was excreted in the urine in 24 hr, but only 50% was excreted by a 3rd patient, when 5-HP was given by a Volutrol. The patient (Case 2) with slow output of urinary radioactivity also showed a much slower fall in plasma levels of radioactivity. This subject experienced considerable vomiting and watery diarrhea during the 1st 2 courses, and fecal excretion amounted to 7% during the 1st course of drug treatment but was only 0.2% of the administered dose in the 2nd. In Cases 2 and 3, the combined vomitus and gastric drainage accounted for 3.6 and 1.4%, respectively, of the administered dose. As was expected, the rate of urinary excretion was slower initially after a 6-hr infusion of drug (26% in 6 and 55% in 24 hr).

Radiolabled metabolites excreted in the urine were studied by ion-exchange chromatography paper chromatography, and thin-layer chromatography; incubation with β-glucuronidase; and by treatment with urease to convert any ^{14}C-labeled urea to $^{14}CO_2$. In all cases, the major urinary component, 41 to 62% of the urinary label, behaved as a glucuronide of 5-HP, while 8 to 20% could be accounted for as unchanged drug. In addition, there was 1 to 18% of a compound, designated "X," which resembled 5-HP in that it could be (*a*) eluted from anion-exchange resin with 0.1 N formic acid and (*b*) extracted with ethyl acetate from neutral aqueous solution. The R_F value of this compound was lower than 5-HP on thin-layer chromatography (0.57, as compared to 0.82 for 5-HP), and its absorption spectrum at pH 9 indicated a λ maximum at 320 nm, as compared to 347 nm for 5-HP. A glucuronide of Compound X accounted for 3 to 15% of the radioactivity. No ethereal sulfates could be detected, but 1 to 5% of the ^{35}S was present as inorganic sulfate.

Measurable amounts of urea, thiourea, or semicarbazide could not be found. No significant amounts of radioactivity were trapped as $BaCO_3$; 100 mg contained 5 to 10 dpm of ^{14}C. These experiments suggest that metabolism of the thiosemicarbazone side chain of 5-HP is negligible in man.

Effects of IQ-1 and 5-HP *in Vitro* on DNA Synthesis in Blood Cells. Initial studies were carried out with IQ-1 in order to determine cell types that might be sensitive to this type of drug. Incorporation of thymidine-^{3}H into DNA was used as a measure of DNA synthesis in both whole blood and isolated white cells. Since red cells do not synthesize DNA, results for uptake studies were based on the white cell count. The results shown in Table 2 are indicative of the inhibitory activity of IQ-1 on thymidine incorporation into DNA. In most cases, isolated white cells were used; with whole blood, the inhibitory activity of IQ-1 appeared to be less pronounced. Cells of chronic lymphocytic leukemia are relatively unaffected by IQ-1. When 5-HP became available, similar screening studies were done. In contrast to results with IQ-1, the use of whole blood or white cell preparations did not affect the degree of inhibition of thymidine incorporation by

Table 1

Levels of 5-HP in the plasma of subjects receiving drug labeled with ^{14}C or ^{35}S

Case	Date	Label	Dosage (mg/kg)	Maximum 5-HP level[a] (μM)[a]	Plasma $t_{1/2}$ (min) Initial	Plasma $t_{1/2}$ (min) Secondary
1		^{14}C	1	13.6	6.8	163
2	8/21	^{35}S	8	145.9	10.5	82
	8/24	^{35}S	4	99.7	6.0	45
	9/11	^{35}S	4	59.2		54
3		^{35}S	16	188.9	2.5	46
		^{14}C	16	216.1	2.5	44
5		^{35}S	8 (i.v. 6 hr)	8.1		123

[a] Levels of 5-HP were determined by column and thin-layer chromatography of deproteinized plasma extracts.

Table 2

Inhibition of the incorporation of thymidine-^{3}H into DNA by human leukemic cells exposed to IQ-1 in vitro

Suspensions of isolated leukemic cells or whole blood were incubated with drug for 15 min before thymidine-^{3}H was added (2 μCi; 6.7 Ci/mmole). Incorporation was then assayed as described under "Materials and Methods."

Cell type	Incorporation as % control at following IQ-1 concentrations 1 μM	2.5 μM	5 μM	10 μM
Acute lymphocytic leukemia	111.6	21.1	7.7	
Acute lymphocytic leukemia	64.0	5.0	3.8	
Acute lymphocytic leukemia	47.6	1.6	0.7	
AML[a]	103.2	12.3	4.3	
AML	89.1	5.4	0.9	
AML[b]		19.9	16.3	8.0
AML[b]		32.3	27.6	
CML	89.3		0.6	0.4
CML	116.2		0.8	0.3
CML	110.3		3.3	1.8
CML	39.6	27.3	1.6	
CLL	91.0	85.9	21.2	
RCS	83.1	5.6	6.8	

[a] AML, acute myelomonocytic leukemia; CML, chronic myelocytic leukemia; CLL, chronic lymphocytic leukemia; RCS, reticulum cell sarcoma.

[b] In these samples, whole blood was used; all other data refer to isolated leukocytes.

Table 3

Inhibition of the incorporation of thymidine-^{3}H into DNA by human leukemic cells exposed to 5-HP in vitro

Whole blood or suspensions of isolated peripheral leukocytes from leukemic subjects were incubated with drug for 15 min at the indicated concentrations, after which thymidine-^{3}H (2 μCi; 6.7 Ci/mmole) was added. The radioactivity in the hot-acid-soluble extract was used to measure incorporation into DNA.

Cell type	Control incorporation (cpm/10^6 cells/0.5 hr)	Incorporation as % control at following 5-HP concentrations				
		5 μM	10 μM	20 μM	50 μM	100 μM
Normal WBC	63	81.6				56.0
Normal WBC	92	72.7		51.2		43.3
Acute lymphocytic leukemia (Case 7)		88.7		79.5		47.9
Acute lymphocytic leukemia (Case 1)	703	96.1	82.1	79.7	49.1	20.1
AML[a]	224	53.4		14.0		10.7
AML	328	58.7		31.1		27.7
AML + Hodgkin's (Case 4)	789	77.6		22.3		8.6
AML	516	69.0	61.4		40.9	13.3
AML	104	112.6		17.4		14.2
AML	381		44.0		29.4	26.8
AML (Case 6)	159	66.9		18.9		8.5
AML (Case 2)	557	43.3		9.5		6.6
CLL	33		81.1		68.9	50.0
CML	752	94.2		15.2		2.9
	753[b]	104.3		15.0		4.0
CML + melanoma	2540[c]	65.6		26.5		10.7
Adenoca rectum (Case 5)	4120[c]	33.7		23.2		4.3

[a] AML, acute myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia.
[b] In this sample, isolated leukemic cells were used.
[c] In these samples, whole marrow suspensions were used.

Table 4

Incorporation of thymidine-^{3}H by peripheral leukocyte suspensions derived from leukemic subjects undergoing treatment with 5-HP

Whole blood was removed before and during treatment with 5-HP and was incubated with thymidine-^{3}H (2 μCi; 6.7 Ci/mmole). Incorporation into DNA was measured as described under "Materials and Methods."

Case	Diagnosis	Dose (mg/kg)	Course	Control incorporation (cpm/10^6 cells/0.5 hr)	Maximum inhibition(%)	Time until recovery (hr)
1	Acute lymphocytic leukemia	1	1	703	61.7	3
		2	2	645	52.9	6
		4	3	743	80.0	
		4(2nd dose)	3	743	84.8	5
		8	4	491	67.8	24
		16	5	280	81.1	24
2	Acute lymphocytic leukemia	8	1	288	88.2	Not determined
		4	2	564	90.0	2
		4	3	1062	84.0	4
4	Acute myelomonocytic leukemia	4	1	1911	62.4	6
		8	2	1350	63.8	6
		8 (2nd dose)	2	1350	65.1	6

5-HP. Most assays were carried out with whole blood, which has advantages in practical terms of fewer manipulations and which most nearly reflects conditions *in vivo*. It would appear (Table 3) that DNA synthesis in normal, mature leukocyte populations and in leukemic cells of the lymphocytic type is least sensitive to 5-HP, while myelocytic leukemic cells, as well as whole normal marrow (*i.e.*, not involved by tumor), are most sensitive to the drug. Incorporation of uridine-^{3}H into RNA was not affected by the concentrations of IQ-1 or 5-HP used.

Effect of 5-HP on DNA Synthesis in Blood Cells *in Vivo*. Since carcinostatic effects of α-(*N*)-heterocyclic carboxaldhyde thiosemicarbazone appears to be correlated with a blockage in the biosynthetic pathway of DNA (1, 4, 5, 15, 16), it was conceivable that optimum drug dosage and scheduling could maintain the effects of 5-HP on this biochemical event in leukemic cells *in situ*. The convenience of using whole blood for assay of thymidine-^{3}H incorporation into DNA made it possible to monitor the effect of drug on DNA synthesis in serial samples. At the lower dosage levels,

incorporation was depressed only for relatively short periods of time. This finding led to a multiple-dose regimen, with administration of the drug every 6 hr. The range of inhibition achieved (52 to 90%) was similar to that obtained by incubating untreated blood *in vitro* with levels of 5-HP comparable to those found in the plasma. In Table 4, data obtained by monitoring changes in thymidine-^{3}H incorporation in 3 leukemic subjects are summarized, while Chart 2 presents the complete course for Case 4. In 2 patients (Cases 1 and 4), there was a decrease in the base line uptake of nucleoside into DNA with successive courses of therapy, while the 3rd showed a rise. These changes presumably reflect alterations in the cell population of peripheral leukocytes. Maximum inhibition was achieved usually about 10 to 30 min after the drug was administered but was not dose dependent. Recovery in most cases occurred within 6 hr or less.

Antineoplastic Effects. No alteration in the progression of advanced metastatic neoplastic disease was seen in any of the 8 patients with solid tumors. Six of these 8 patients demonstrated some hematological effects, such as modest myelosuppression or hemolysis, after drug treatment was initiated. Three of 5 patients with acute leukemia demonstrated transient decreases in blast cell counts. The dosage schedules used and the brief responses seen are summarized in Table 5. The 1st patient was treated with intermittent doses during a 7-week period, and transient decreases in blast count were seen after each dose, although an overall increase in peripheral blasts occurred. With experience, shorter courses of therapy with higher doses were used. The best effects observed with 5-HP were achieved in Case 4. Three days of therapy during a 2-week period produced a progressive fall in blasts; however, unfortunately, the patient succumbed to infection during the subsequent period of hypoplasia (Chart 2). Case 2 experienced an approximately 30% reduction in lymphadenopathy, which persisted for 1 week in association

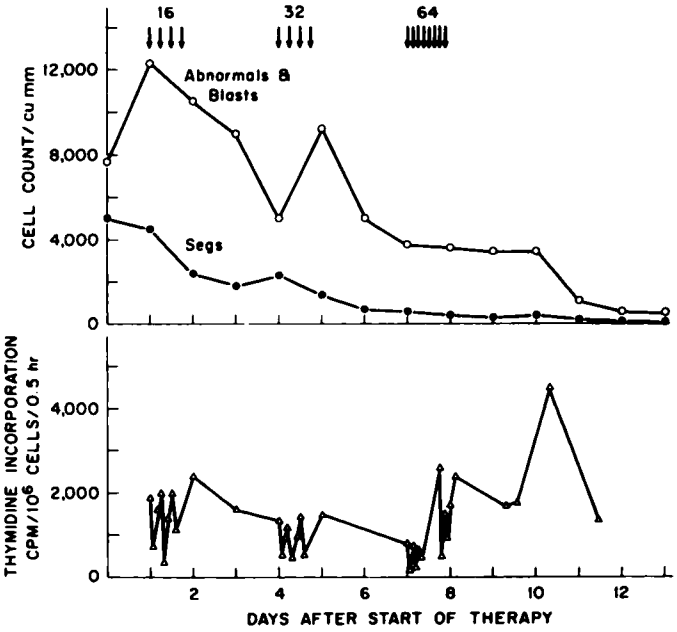

Chart 2. Effects of treatment with 5-HP on peripheral leukocyte and abnormal cell counts and their thymidine-incorporating activity in Case 4. Details of the assay system for thymidine-^{3}H incorporation are given in "Materials and Methods."

Table 5

Dosage schedules and antineoplastic effects of 5-HP in patients with acute leukemia

Case	Age	Sex	Diagnosis	Dosage and frequency of administration (mg/kg × daily doses)	Duration of treatment period	Maximal therapeutic effect	Subsequent course
1	15	F	Acute lymphocytic leukemia	1 × 1 2 × 2 4 × 2 4 × 4 8 × 2 8 × 4 16 × 1	A total of 7 wk	Transient falls in blasts 4,000 → 2,000 30,000 → 6,000	Blast proliferation despite increasing drug dosages. No response to methotrexate Expired with pseudomonas sepsis
2	21	F	Acute lymphocytic leukemia	8 × 4 4 × 4 Given 7 × increasing to every other day	3 wk	Transient falls in blasts 18,000 → 5,000 20,000 → 4,000 30% reduction in adenopathy	Blast proliferation. Complete remission induced with asparaginase.
4	28	F	Acute myelomonocytic leukemia	4 × 4 8 × 4 8 × 8	2 wk	Progressive falls in blasts	Expired with pneumonia and sepsis
6	40	M	Acute myelomonocytic leukemia	16 mg/kg/24 hr infusion × 5 days 16 mg/kg/6 hr infusion	1 wk	None	Blast proliferation during drug treatment period 20,000 → 76,000. Hypoplasia achieved with combined therapy with cyclophosphamide, methotrexate, and cytosine arabinoside
7	38	M	Acute lymphocytic leukemia	32 mg/kg/24 hr infusion × 5 days	2 wk	Transient fall in blasts 62,000 → 31,000	Rapid blast proliferation arrested by adriamycin

with a decrease in peripheral blasts, but the patient relapsed while continuing to receive drug. Two patients received 5-HP by infusion in an attempt to avoid the gastrointestinal effects of the drug; antileukemic activity was not appreciably different. Some response to 5-HP in patients with acute myelocytic and lymphocytic leukemia was achieved with these abbreviated treatments. The treatment period was kept brief deliberately to avoid undue risks, and neoplastic cell proliferation after a 5-day course of therapy was considered evidence of drug failure.

DISCUSSION

The toxicity observed in the initial trials of 5-HP in man correspond closely to that noted in preclinical studies. Thompson *et al.* (21) demonstrated in dogs that doses of 5 mg/kg produced vomiting, diarrhea, transient leukopenia, and a dark green urine. A comparison of injections with infusions of 5-HP in monkeys demonstrated that the frequency of vomiting decreased when the drug was given by prolonged i.v. infusion. These findings correspond closely to those of this study, in which nausea, vomiting, and diarrhea were evident at doses above 4 mg/kg, and it appeared that prolonged i.v. infusion ameliorated these effects. Renal tubular necrosis was not seen at the doses used in man. This study provides an explanation for the intense green urine, which in man and presumably in animals represents increased excretion of iron. The increased phosphaturia observed in 5 patients may reflect a more subtle renal tubular lesion. A biphasic curve for drug clearance was observed for 5-HP, with a $t_{1/2}$ ranging from 2.5 to 10.5 min during the early rapid phase of drug disappearance from plasma. This is somewhat shorter than the $t_{1/2}$ of 15 min observed in mice (22).

The metabolic fate of 5-HP as evaluated in this study presents some interesting contrasts to what is known of the metabolism of thiosemicarbazones in other species. Desulfuration to give the semicarbazone, with release of inorganic sulfate, is the major pathway for the degradation of 1-methylisatin 3-thiosemicarbazone (17) and of IQ-1 by dogs and mice (6, 7). In the case of 5-HP, desulfuration is a minor process. Conjugation to form glucuronides (50 to 74% of excreted radioactivity) apparently is the major metabolic fate of 5-HP, in contrast to IQ-1, of which only approximately 13% is excreted in this form (6). No evidence could be found for degradation of the thiosemicarbazide portion of 5-HP, although products of degradation of the side chain have been demonstrated with IQ-1 (6, 7). That this latter difference is not merely a species difference but the reflection of contrasting metabolic susceptibilities of the compounds themselves is suggested by our finding that release of $^{14}CO_2$ in expired air from 5-HP-3'-^{14}C is 10 to 20 times lower in the mouse than is release from IQ-1-3'-^{14}C.

This study demonstrated that, *in vivo* and *in vitro*, 5-HP inhibited DNA synthesis in a variety of leukemic leukocytes. It was disappointing to note that the inhibition of the formation of DNA noted in cells *in vitro* after exposure to 5-HP *in vivo* was only transient despite a wide range of dose levels used. It had been expected that, as the number of doses of 5-HP was increased, or when the compound was given by 24-hr infusion, a sustained inhibition of DNA synthesis would be realized and a simulation of the optimum therapeutic schedules of Skipper *et al.* (19) would be obtained. This was not achieved. Evidently, high concentrations of 5-HP could not be maintained because of the extremely rapid clearance of drug during the 1st 10 to 20 min after administration. In addition, a significant fraction of the declining plasma drug level represents protein-bound material. These factors would tend to make 5-HP unavailable in adequate amounts at its site of action on ribonucleoside diphosphate reductase within the tumor cells. Further, 5-HP has a lower affinity for the enzyme than other agents of this class, such as IQ-1 (2, 16).

The toxicity observed at doses of 4 mg/kg and greater may be sufficient to prohibit further studies of the drug as an antineoplastic agent. The observed gastrointestinal toxicity deserves some discussion with regard to the factors responsible for it. Preclinical studies of the distribution of IQ-1 in mice have shown that the intestine may retain up to 40% of the administered radioactivity over a 24-hr period, and the stomach may retain up to 6% (6, 7). Similar experiments with labeled 5-HP showed that mouse intestine may contain about 30% of the total administered dose. The iron stores associated with the gastrointestinal tract may be responsible for retention of these chelating thiosemicarbazones, and the concentration of these agents in the gut could be responsible for the observed nausea, vomiting, and diarrhea. In 1 patient (Case 2), the initial toxicity was very acute but diminished in intensity after several courses. This could be correlated with more rapid initial rates of plasma clearance and urinary excretion during the later course of therapy.

The marked enhancement of iron excretion in chelated form observed after 5-HP administration suggests that further studies with this drug or similar thiosemicarbazones in disease states such as hemachromatosis or thalassemia, in which iron overload may pose grave clinical problems, may be warranted. Furthermore, the development of a 2nd-generation clinical agent of this class with greater affinity for the target enzyme, ribonucleoside diphosphate reductase, and other selective enzymes in the biochemical pathway for DNA biosynthesis should be considered; such agents may produce less gastrointestinal toxicity and be better tolerated in man.

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