The Carcinostatic Activity of α-(*N*) Heterocyclic Carboxaldehyde Thiosemicarbazones

I. Isoquinoline-1-carboxaldehyde Thiosemicarbazone[1]

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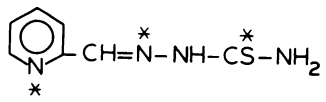
SUMMARY

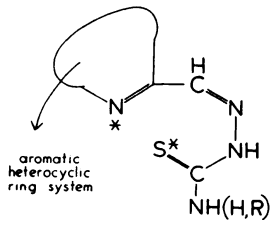
Isoquinoline-1-carboxaldehyde thiosemicarbazone (IQ-1) has been shown to have carcinostatic activity against leukemias L-1210 and ML-1210, the Lewis lung carcinoma, Adenocarcinoma 755, and the Ehrlich ascites carcinoma. IQ-1[2] is active, in some instances, by both oral and parenteral routes and with delayed treatment. This compound is a member of a broad class of α-(*N*) heterocyclic carboxaldehyde thiosemicarbazones, several of which have demonstrable carcinostatic activity. The common feature of these compounds is that they contain an N*—N*—S* tridentate ligand system.

In 1956 Brockman *et al.* (2) reported on the anti-leukemic effect of pyridine-2-carboxaldehyde thiosemicarbazone (I). This activity was verified in our laboratory. Notable is the fact that this compound is treacherously and cumulatively toxic. The single LD_{50} is approximately 40 mg/kg i.p. Later we studied a number of the more readily accessible heterocyclic carboxaldehyde thiosemicarbazones and found no encouraging degree of activity (3, 5, 6). This area appeared unpromising.

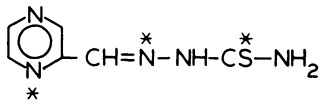
Late in 1963 we surveyed and reanalyzed the available data on simple hydrazones, including mono(thiosemicarbazones), and formulated a set of hypotheses that were testable by experiment. The basic hypothesis was that pyridine-1-carboxaldehyde thiosemicarbazone was acting as a tridentate ligand with a predilection for forming octahedral coordination compounds (chelates) with divalent ions on the right-hand side of the 1st transition series and also heavier transition elements, such as cadmium. This hypothesis may be conveniently summarized by a general formula (II). The *asterisked points* are the points of attachment to metal ions in coordination compounds. The 2nd basic hypothesis was that modifying the ring system while retaining the ligand pattern could lead to improved activity and decreased toxicity. It was also postulated that π and σ electron densities, substituents, and details of geometry would have critical effects. A number of predicted negative compounds were made for the purpose of



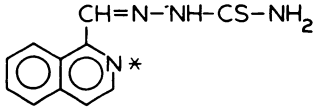
I



II



III



IV

sharply delineating the territory of interest. The thiosemicarbazones of the following carboxaldehydes were

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[2] The abbreviations used are: IQ-1, isoquinoline-1-carboxaldehyde thiosemicarbazone; LD_{50}, median lethal dose; LLC, Lewis lung carcinoma; KTS, 3-ethoxy-2-ketobutyraldehyde bis(thiosemicarbazone).

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predicted and found negative on leukemia L-1210, Adenocarcinoma 755, Sarcoma 180, and the Lewis lung carcinoma: furan-2-; 5-methylfuran-2-; thiophene-2-; pyrrole-2-; *N*-methylpyrrole-2-; indole-2-; *vic*-triazole-; imidazole-4(5)-; pyrazole-3-; cinnoline-3-; benzothiazole-2-; quinoline-8-; quinoxaline-2-; 3-hydroxyquinoxaline-2-; and 3-methoxyquinoxaline-2-. Some of these compounds are toxic and also occasion significant weight loss without significant effects on the tumors. The 1st predicted active compound was pyrazine carboxaldehyde thiosemicarbazone (III). The anti-tumor and *in vivo* chelating ability of this compound have been reported (8).

In our laboratory we found the pyridine derivative (I) moderately active on L-1210, active on the Lewis lung carcinoma only at the expense of excessive weight loss, and inactive on Adenocarcinoma 755 and the Ehrlich ascites carcinoma at tolerated levels. The pyrazine derivative (III) was more active on L-1210, active on 755 and LLC, but ineffective on the Ehrlich carcinoma. Next we predicted and found IQ-1 (IV) active and the isoquinoline-3 derivative inactive in our tumor systems. IQ-1 is more broadly and intensely active than the previous compounds; it is also less toxic. Additional activities corresponding to the general formula (II) have been found and will be reported later when the data are complete. The area indicated by Formula II constitutes a new class of carcinostatic agents.

MATERIALS AND METHODS

Chemistry.—Isoquinoline-1-carboxaldehyde was prepared by the method of Barrows and Lindwall (1). The thiosemicarbazone was prepared as follows: 18 gm of the aldehyde was dissolved in 110 ml of ethanol. Twelve gm of thiosemicarbazide was dissolved in 70 ml of water and 2 ml of acetic acid. The hot solution of the aldehyde was added to the thiosemicarbazide solution. Separation of the product crystals started in 10 sec. After being cooled, the product was filtered and washed with water, alcohol, and ether. Purification was best effected by 2 thorough hot water washes alternated with 2 thorough hot ethanol washes. The yield was 23.0 gm (87.2%); m.p., 224°–226°C (decomp).

Analytical data.—Calculated for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33; S, 13.92. Found: C, 57.20; H, 4.50; N, 24.48; S, 14.09.

Biology.—The mice used in these experiments were ob-

TABLE 1

EFFECT OF ISOQUINOLINE-1-CARBOXALDEHYDE THIOSEMICARBAZONE ON L-1210 LEUKEMIA IN MICE

There was no early mortality in any experiment. Ten mice were used in each experimental group and in the controls.

DRUG DOSAGE (mg/kg or % in diet)	ROUTE	DAY TREATMENT STARTED[a]	NO. OF DAYS TREATED	% WT. CHANGE (4 days)	MEAN SURVIVAL TIME (days)[b]		% INCREASE	*P* VALUE
				Treated/control	Treated	Control		
20	i.p.	1	8	−3/+7	10.3 ± 0.4	7.7 ± 0.3	34	0.00006
				+1/+14	9.9 ± 0.6	7.4 ± 0.2	34	0.00007
40	i.p.	1	8	−4/+7	11.6 ± 0.6	7.7 ± 0.3	51	0.00002
				−3/+2	11.4 ± 0.3	7.4 ± 0.2	54	<0.00001
67	i.p.	1	8	−3/+16	10.6 ± 0.2	7.2 ± 0.1	47	<0.00001
				−8/+6	11.8 ± 0.4	7.8 ± 0.3	51	<0.00001
80	i.p.	1	8	−11/+16	11.4 ± 0.3	7.2 ± 0.1	58	<0.00001
				−14/+2	12.5 ± 0.9	7.4 ± 0.2	69	0.00002
		3	6	+2/+9	9.8 ± 0.2	7.0	40	<0.00001
				+1/+16	10.6 ± 0.3	7.2 ± 0.1	47	<0.00001
		4	5	+10/+9	10.1 ± 0.1	7.0	44	<0.00001
				+13/+16	10.9 ± 0.4	7.2 ± 0.1	51	<0.00001
		5	4	+13/+9	10.4 ± 0.5	7.0	49	<0.00001
				+9/+16	8.8 ± 0.2	7.2 ± 0.1	22	0.00002
113	Gavage	1	6	−9/+6	9.6 ± 0.3	7.2 ± 0.1	33	<0.00001
				−8/+13	10.6 ± 0.9	7.4 ± 0.2	43	0.003
0.25%	Diet	1	cont[c]	−10/+10	10.2 ± 0.8	7.1 ± 0.1	44	0.001
				−13/+12	11.1 ± 0.7	7.6 ± 0.2	46	0.00006

[a] Tumor inoculated on Day 0.

[b] ±S.E.

[c] Diet continued until death of all animals.

tained from Simonsen Laboratories, Gilroy, California and maintained on Purina chow. The tumors were procured from Microbiological Associates, Washington, D. C. In leukemias L-1210 and ML-1210 [resistant to methylglyoxal bis(guanylhydrazone)] C57BL x DBA F_1 (hereafter called BDF_1) mice were inoculated i.p. with 10^6 leukemia cells. The % increase in mean survival time of the treated animals relative to the controls was the evaluation index. In Adenocarcinoma 755 and LLC, BDF_1 mice were given s.c. inoculations in the groin of 0.2 ml of a suspension of crushed tumor tissue in normal saline (200 mg/ml). These experiments were terminated in 11 days, and the tumors were excised and weighed individually to a precision of ±1 mg. The index of effectiveness was % inhibition. Sarcoma 180 and the Ehrlich ascites carcinoma were evaluated in Swiss mice. In the S-180 experiments the mice were given inoculations in the groin of 0.2 ml of a suspension of tumor tissue in normal saline (200 mg/ml). The experiments were terminated in 7 days and evaluated as for Adenocarcinoma 755. In the Ehrlich carcinoma experiments the mice were given i.p. injections of 10–20 million undiluted tumor cells. No attempt was made to standardize the inoculum, but cell counts were made in each experiment. The index used was the same as for L-1210. Gavage, i.p., and diet routes of drug administration were explored. In some instances the effect of late treatment was studied. Standard errors and *P* values for the individual experiments were calculated by means of Student's statistics.

RESULTS

The results with leukemia L-1210 are given in Table 1. These are typical values obtained in experiments too numerous to include. Best results by the i.p. route are obtained in the 40–80 mg/kg range although activity is evident at lower levels. Higher doses are toxic. Of greater interest are the results of delayed therapy. If drug treatment is delayed 3, 4, or 5 days after tumor inoculation, significant activity remains. The drug is also active p.o. IQ-1 shows comparable activity on ML-1210. This is an L-1210 made resistant to methylglyoxal bis(guanylhydrazone) that has been carried in this laboratory for many years (7).

The results of testing IQ-1 on the Lewis lung carcinoma are presented in Table 2. Response is good by diet, gavage, or i.p. routes of administration. It may be noted that in 4 experiments a significant proportion of the mice had no discernible tumor at autopsy. Good inhibition was obtained even when drug administration was delayed to the 4th or 7th day after tumor inoculation.

IQ-1 is also active on Adenocarcinoma 755. In Table 3 one may note that inhibitions in the 70–80% range are readily obtainable. Delayed therapy was not effective, in contrast to the results on L-1210 and LLC.

One notes in Table 4 that IQ-1 is quite active on the Ehrlich ascites carcinoma when given i.p. When treatment is delayed to the 3rd day some activity is retained, but a further delay to the 5th day abolishes activity. Oral routes are ineffective.

The activity on Sarcoma 180 is at best marginal and generally accompanied by toxicity.

Detailed toxicologic and pharmacologic investigations are pending. However, brief studies have been conducted. Single i.p. doses up to 400 mg/kg are well tolerated by 20-gm female Swiss mice. The single (i.p.) LD_{50} is approxi-

TABLE 2

EFFECT OF ISOQUINOLINE-1-CARBOXALDEHYDE THIOSEMICARBAZONE ON THE LEWIS LUNG CARCINOMA IN MICE

Ten mice were used in each experimental group and in the controls.

Drug dosage (mg/kg or % in diet)	Route	Day treatment started[a]	No. of dasy treated	% wt. change (11 days)	No. surviving	Mean tumor wt. (mg) (11 days)[b]		% inhibition
				Treated/control		Treated	Control	
67	i.p.	1	9	−10/+1	10	105 ± 40	862 ± 95	88
				−4/+11	10	257 ± 33	1231 ± 80	79
80	i.p.	1	9	−23/+9	7	0[c]	767 ± 69	100
67	Gavage	1	9	−5/+11	10	196 ± 30	1231 ± 80	84
				−11/+1	10	56 ± 20	862 ± 95	94
80	Gavage	1	9	−11/+9	9	22 ± 4	767 ± 69	97
				−13/+1	10	24 ± 10[d]	862 ± 95	97
113	Gavage	1	9	−14/+9	6	0[e]	767 ± 69	100
80	Gavage	4	6	−9/+5	10	36 ± 10	531 ± 102	93
		7	4	−7/+1	10	289 ± 62	862 ± 95	66
				−6/+5	10	90 ± 21	531 ± 102	83
0.05%	Diet	1	10	0/+5	10	88 ± 12	531 ± 102	83
0.1%	Diet	1	10	−8/+5	10	33 ± 8	531 ± 102	94
				−9/+11	10	85 ± 30	1231 ± 80	93
0.15%	Diet	1	10	−22/+1	10	34 ± 15[f]	862 ± 95	96

[a] Tumor inoculation on Day 0.
[b] ±S.E.
[c] Three toxic deaths; the remaining 7 mice had no tumors.
[d] Five mice had no tumors.
[e] Four toxic deaths; the remaining 6 mice had no tumors.
[f] Four mice had no tumors.

TABLE 3

EFFECT OF ISOQUINOLINE-1-CARBOXALDEHYDE THIOSEMICARBAZONE ON ADENOCARCINOMA 755

Ten mice were used in each experimental group and in the controls.

DRUG DOSAGE (mg/kg or % in diet)	ROUTE	DAY TREATMENT STARTED[a]	NO. OF DAYS TREATED	% WT. CHANGE (11 days) Treated/control	NO. SURVIVING	MEAN TUMOR WT. (mg) (11 days)[b] Treated	Control	% INHIBITION
67	i.p.	1	9	−10/+9	10	210 ± 103	1088 ± 198	81
				−11/+11	10	178 ± 52	1851 ± 337	90
80	i.p.	1	9	−28/+14	7	25 ± 5	1645 ± 109	98
57	Gavage	1	9	−4/+7	10	205 ± 62	839 ± 204	76
				−7/+13	10	505 ± 91	2197 ± 340	77
80	Gavage	1	9	−14/+9	9	114 ± 73	1088 ± 198	90
				−12/+8	10	56 ± 26	712 ± 189	92
0.05%	Diet	1	10	+4/+7	10	256 ± 114	877 ± 200	71
				0/+8	10	212 ± 88	712 ± 189	70
0.1%	Diet	1	10	−10/+8	10	29 ± 12[c]	712 ± 189	96
				−16/+9	10	21 ± 5	1088 ± 198	98

[a] Tumor inoculation on Day 0.
[b] ±S.E.
[c] Five mice had no tumors.

TABLE 4

EFFECT OF I.P. ISOQUINOLINE-1-CARBOXALDEHYDE THIOSEMICARBAZONE ON THE EHRLICH ASCITES CARCINOMA

There was no early mortality in any experiment. Ten mice were used in each experimental group and in the controls.

DRUG DOSAGE (mg/kg)	DAY TREATMENT STARTED[a]	NO. OF DAYS TREATED	% WT. CHANGE (4 days) Treated/control	MEAN SURVIVAL TIME (days)[b] Treated	Control	% INCREASE	*P* VALUE	NO. SURVIVING 60 DAYS
10	1	11	+10/+24	21.7 ± 2.4	8.5 ± 0.9	155	0.0001	
			+6/+27	17.2 ± 1.3	8.8 ± 1.6	95	0.0001	
20	1	11	0/+22	21.9 ± 2.1	10.1 ± 1.7	117	0.0004	1[c]
			−4/+16	21.5 ± 1.0	8.9 ± 0.8	142	<0.00001	
40	1	11	−4/+16	24.7 ± 1.5	8.9 ± 0.8	178	<0.00001	
			0/+22	25.4 ± 4.8	10.1 ± 1.7	151	0.005	2[d]
57	1	11	−7/+14	27.0 ± 3.5	9.6 ± 0.3	181	0.00008	2[e]
			−5/+16	25.6 ± 4.7	8.9 ± 0.8	188	0.003	
67	1	11	−3/+14	30.7 ± 4.7	9.6 ± 0.3	220	0.0003	
			−10/+22	16.8 ± 3.1	10.1 ± 1.7	66	0.04	
57	3	9	+9/+14	19.3 ± 2.8	9.6 ± 0.3	101	0.005	
			+15/+24	16.0 ± 2.9	8.5 ± 0.9	88	0.02	1[c]

[a] Tumor inoculation on Day 0.
[b] ±S.E.
[c] Tumor free.
[d] One mouse had a solid tumor.
[e] Both mice had solid tumors.

mately 800 mg/kg. Accurate determination is hampered by the difficulty of introducing large amounts of drug suspension and uncertainties in the rate of absorption.

Brief pathology studies were conducted. Normal BDF_1 mice given 8 daily i.p. injections of 67 mg/kg and sacrificed on the 10th day showed no significant abnormalities of the stomach, small and large intestine, heart, lungs, liver, spleen, lymph nodes, kidney, pancreas, adrenals,

femoral bone marrow, and blood from the posterior vena cava. This dose and schedule are well tolerated and close to optimum for L-1210, AC-755, and LLC.

Untreated BDF_1 mice bearing L-1210 leukemia were autopsied on the 7th day after tumor inoculation. The same tissues were examined. There was severe generalized infiltration of tissues with highly malignant small mononuclear cells. This was especially prominent in retroperitoneal tissues, fat, pancreas, liver, spleen, and lymph nodes. The bone marrow preparations showed numerous immature cells, including many blast forms. The blood revealed frequent immature blast forms and sparse platelets.

In contrast, leukemic mice receiving 5 daily intraperitoneal treatments (67 mg/kg) of IQ-1, and sacrificed on the 7th day, showed less invasion of retroperitoneal tissues, including the liver. The spleen was relatively spared, and the thymus was slightly depleted. Except for the infiltration, the tissues were within normal limits. In the blood, platelets were abundant and vacuolated blast forms were infrequently noted. The bone marrow preparations were relatively normal, although blast forms were present but not unusually prominent.

Another group of leukemic mice were given 8 daily i.p. drug treatments (67 mg/kg) and sacrificed on the 10th day. The infiltration by tumor cells was much heavier and more generalized than in the 7-day sacrifice group. Heavier infiltration of the spleen and pulmonary capillaries was noted. The blood picture was still predominantly normal. Some vacuolated blast forms were noted. The bone marrows still showed numerous normal elements, but primitive cells were frequently noted.

We are attempting to produce a line of L-1210 that is resistant to IQ-1, but no resistance has appeared after 17 transplant generations.

DISCUSSION AND CONCLUSIONS

It is intersting that IQ-1 is active on 5 out of the 6 tumors we carry in our laboratory. In order to more clearly define its range of biologic activity, IQ-1 and related compounds have been sent to numerous laboratories, and sundry biologic and chemical studies are in progress.

In Formulas I, III, and IV one may note that the N*—N*—S* tridentate ligand system is present in each molecule. The 2 resultant chelate rings for each ligand would be 5-membered, highly conjugate, and hence tending toward high stability unless there is interference from steric and strain effects. In case all 3 groups in the ligand are utilized the resultant chelate, ML_2, would be neutral if the metal ion, M, is divalent. Qualitative tests in this laboratory show that I, III, and IV form intensely colored complexes with divalent iron, copper, nickel, cobalt, and zinc at neutral or alkaline pH values. The ferrous chelates are electrically neutral, supporting the tridentate hypothesis. Specificity of this type of ligand would be directed toward divalent cations with an octahedral coordination habit and particularly those that are azophiles and sulfurophiles. Quantitative studies of these ligands are being conducted by a collaborating laboratory.

We discussed the metal chelation hypothesis earlier in relation to the anti-tumor activity of the *vicinal* bis(thiosemicarbazones) (4). Recent work by Petering and coworkers (9, 10) has shown that cupric ion is required as an activator for KTS and that zinc mitigates toxicity. The bis(thiosemicarbazones) have an entirely different ligand system: S*—N*—N*—S*. They are tetradentate and tend to form 1:1 square, coplanar complexes with certain divalent cations. Hence I, III, and IV fall into a class different from KTS. This is exemplified *in vivo* by their different anti-tumor spectra in mice. In our laboratory there is 1 instance of overlap. KTS, III, and IV are active on the Lewis lung carcinoma.[3] This should be an interesting point for study.

In vitro chelation systems are very simple compared to what can occur *in vivo*. In the living system there are numerous micro- and macromolecular structures that contain potent ligands, and these may be expected to participate in the exotic drug-natural ligand-metal ion interactions. Details of molecular structure relatively unrelated to ligand capabilities *per se* may be expected to play crucial roles. Experience in this and other laboratories with both classes of thiosemicarbazones shows that this is the case.

Nothing definite can be said about the mechanism of action of IQ-1 at this time. The metal chelation hypothesis is tempting and has proven fruitful as a tool for drug development. So far it has been unilluminating at the actual mechanism level. This is partly because of the lack of an adequate relevant body of experimental work. It is to be hoped that further work will yield the necessary insight and better drugs.

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[3] This laboratory, unpublished data.