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Anti-Cyanide Drugs

Annual Report

Peter Hambright

September 1, 1989

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

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Department of Chemistry
Howard University
Washington, D. C. 20059

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The purpose of this project was to identify and develop prophylactic anti-cyanide agents including metal complexes, alkylidines, metalloporphyrins and aldehyde and ketone cyanohydrin formers. The screen involved pre-administration of drugs to mice at 15 and 60 minutes, followed by 2 LD-50s of KCN. Out of forty-three drugs, only nickel(II) chloride, cobalt(II)-chloride and 2-carboxybenzaldehyde survived the screen. The affinity of lower oxidation states of cyanocobalt(III)porphyrins and metal-EDTA adducts for cyanide was probed, and complexes that selectively release a cyanide specific metal upon demand were investigated.

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SUMMARY

The purpose of this project was to develop drugs that will act in a prophylactic fashion in vivo against the potential CW agent, cyanide. Molecules thought to have potential as cyanide binders were to be investigated for their cyanide uptake ability in solution around the physiologic pH of 7.4. The best derivatives were to be submitted to WRAIR for further testing. The species were to be classical metal ion complexes, and metal ions bound to water soluble porphyrins and phthalocyanines. In addition alkylidenes, species containing double bonds activated by electron withdrawing groups, were investigated for their capacity of adding cyanide across the double bond. Cyanohydrin formers such as aldehydes and ketones were also explored.

We received the results on forty-three compounds tested in a cyanide screen by Army. In general, 2LD-50s of cyanide were administered to mice that had been pre-treated with certain levels of drugs either 15 minutes or 60 minutes prior to the cyanide, and the number alive after 24 hours were noted. The control mice were given nitrite/thiocyanate combinations 60 minutes before cyanide challenge. At least four out of ten mice had to survive the challenge for the compounds to be considered as having PASSED the screen. Four dose levels were tested for each pre-administration time period, and such doses were generally 1/4, 1/16, 1/32 and 1/64 of the maximum toxic LD-50 of the drug itself. Three compounds PASSED the screen, NiCl2.6H2O, CoCl2.6H2O and 2-carboxybenzaldehyde. Two other compounds, 4-pyridine- carboxaldehyde-N-oxide and ethyl-alpha-cyclohexlidene-alpha-cyanoacetate were still active, as they had LD-50s of 1000mg/kg body weight, and insufficient amounts were on hand for further testing. Little correlation was found between the in vitro and in vivo binding results.

A pulsed radiolysis and cyclic voltammetry study on the reduction of cyano-cobalt(III) porphyrins indicated that some complexes were reduced at the metal center, while others added electrons only to the porphyrin ring. The Co(II) states were still complexed with cyanide, whereas all cyanide binding ability was lost at the Co(I) level.

We showed the effect of EDTA complexation of cyanide uptake by aquo Ni(II) and Co(II) ions. In general, addition of EDTA forming M(EDTA)2- species reduces the cyanide complexing ability of the metal. While Co(II) adds five and Ni(II) ions four moles of cyanide, the M(EDTA) species bind only about 0.1 mole at pH 7.4. The Co[Co(EDTA] takes up six moles of cyanide, perhaps five from the outer Co(II) and one from the inner EDTA chelated metal.

We began developing compounds that would release a toxic metal only in the presence of cyanide. Thus, stable Ni(II) derivatives of central N-methylated water soluble porphyrins react with four moles of cyanide and Ni(CN)4(2-) is rapidly released to solution. This nickel dissociation stops upon depletion of the cyanide present.

TABLE OF CONTENTS

COMPOUNDS SUBMITTED

NO.	NAME	WR#
A-89	Ethyl-alpha-cyclohexylidine-alpha-cyanoacetate	11100AF
B-89	4-pyridinecarboxaldehyde-N-oxide	133059AB
C-89	Nickel(II)-ethylenediaminetetra-acetic acid, 0.1 M solution	
D-89	Cobalt(II)-ethylenediaminetetra-acetic acid, 0.1 M solution	
E-89	Di-cobalt(II)-ethylenediamine-tetraacetic acid, 0.1 M solution	
F-89	Tetraethylammonium bis-maleonitrile-nickelate(II)	
G-89	Hydroxocobalamin hydrochloride	
H-89	Chloro(pyridine)cobaloxime(III)	
I-89	Sodium hexanitrocobaltate(III)	
J-89	Auranofin	
K-89	Chlorotriethylphosphinegold(I)	
L-89	Chloro-bis-triethylphosphinegold(I)	
M-89	Sodium aurothiomalate monohydrate	
N-89	Glyoxal, 40% by wt solution	
O-89	Acetaldehyde, 40% by wt solution	

COMMENTS ON SUBMITTED COMPOUNDS

A-89. Ethyl-alpha-cyclohexylidine-alpha-cyanoacetate. WR # 11100AF. We resubmitted 25 gms of this compound upon request, as it had an LD-50 of over 1000 mg/kg, and may have some anti-cyanide action. The compound was synthesized based on literature methods [1],

B-89. 4-Pyridinecarboxaldehyde-N-oxide. WR # 133059AB. We resubmitted five grams of this compound upon request, as its LD-50 was over 1000 mg/kg in mice, and may have anti-cyanide properties. The compound was purchased from Aldrich Chemicals, and was recrystallized and analyzed. Using the cyanide electrode, it rapidly bound 0.82 moles of cyanide per mole of compound at pH 7.4.

C-89. Nickel(II)-ethylenediaminetetraacetic acid, 0.1 M solution. Using a cyanide specific electrode, aquo Ni(II) picks up four moles of CN, whereas Ni(EDTA) reacts with only 0.14 moles. The solution was prepared from analyzed NiCl2.6H2O and disodium ethylene-diaminetetraacetic acid.

D-89 Cobalt(II)-ethylenediaminetetraacetic acid, 0.1 M solution. About 0.1 mole of cyanide was bound per mole of Co with the presumed less toxic Co(II)-EDTA species, which was prepared from analyzed CoCl2.6H2O and Na2-EDTA. In contrast, aquo Co2+ binds five moles of cyanide at pH 7.4.

E-89 Di-cobalt(II)-ethylenediaminetetraacetic acid, 0.1 M solution. This Co2-EDTA binds 6.0 moles CN per mole of EDTA. It was prepared from analyzed CoCl2.6H2O and Na2-EDTA, and is a known in-vivo cyanide scavenger [2].

F-89 Tetraethylammonium bis-maleonitrile-nickelate(II). This compound was synthesized by literature methods [3]. Upon standing in base with cyanide, the species decomposes, and binds four moles of cyanide per mole of nickel. In addition, the drug contains sulfur which may act as a substrate for the SCN forming enzymes.

G-89 Hydroxocobalamin hydrochloride. The cobalt(III) Vitamin B-12 derivative was found to bind 2.0 moles of cyanide, and is a recommended anti-cyanide agent [4]. It has a low toxicity of 2g/kg (mouse i.v.). However, massive amounts of hydroxocobalamin need to be administered (ca 1400 mg of hydroxocobalamin neutralize 28 mg of cyanide), and while it rapidly reacts against

cyanide *in vivo*, its action also rapidly abates at longer times [5]. The compound was purchased from Sigma and analyzed.

H-89 Chloro(pyridine)cobaloxime(III). The compound was synthesized by known methods [6], and should act in a manner similar to Vitamin B-12. It has a formula weight of 403 daltons, one-third that of B-12.

I-89 Sodium hexanitrocobaltate(III). This compound was purchased from Aldrich Chemicals and analyzed. It has shown anti-cyanide activity in sheep [7].

J-89 Auranofin. This gold(I) compound is oral antiarthritic drug from SK&F, and in previous work [8] was shown to bind one mole of cyanide per mole of substrate.

K-89 Chlorotriethylphosphinegold(I). The compound was on loan from Dr. S. Baskin (ICD), who purchased it from Strem Chemicals, and we had it analyzed. It might have cyanide affinity, as a slurry in water showed cyanide uptake properties, but it is rather insoluble.

L-89 Chloro-bis-triethylphosphinegold(I). This compound was on loan from Dr. S. Baskin (ICD), who purchased it from Strem Chemicals, and we had it analyzed. This gold(I) compound bound 0.64 moles CN per mole of gold, at pH 7.4.

M-89 Sodium aurothiomalate monohydrate. This species was purchased from Aldrich Chemicals, and analyzed. It bound 2.0 moles of cyanide per mole of gold, and is also an antiarthritic drug.

N-89 Glyoxal, 40% by wt solution. Glyoxal is a water soluble cyanohydrin forming cyanide scavenging agent, and binds 0.6 moles of cyanide per formula weight of substrate. It was purchased from Aldrich Chemicals.

O-89 Acetaldehyde, 40% by wt solution. Acetaldehyde (Aldrich Chemicals, 99%) was made 40% by weight in water in the cold room. It is the lowest molecular weight cyanohydrin former, and was found to bind one mole of CN per mole of substrate at pH 7.4. Thus, 44mg of CH_3CHO complexes 28 mg of cyanide *in vitro*.

GENERAL RESULTS

The aim of this project was to find molecules that would prophylactically protect man against the rapid acting poison, cyanide. The classes of compounds to be investigated included metal ions and metal complexes which would form cyanide adducts, and aldehydes and ketones which could act as cyanohydrin formers. In addition, we were interested in alkylidenes, organic molecules containing a double bond one end of which is attached to a strongly electron withdrawing species. This polarization favors addition of cyanide to the incipient carbonium ion. The compounds should be water soluble, and show in vitro a substantial cyanide affinity at in physiologic pH range near pH 7.4.

Forty-three compounds that we submitted to WRAIR as anti-cyanide agents have been screened in a mouse model, and the results became available this year. The work was done at the Medical Research and Evaluation Facility of Battelle, in Columbus, Ohio, in contract with the ICD. The acute toxicity of the drugs in ca 25 gram male albino mice (from Charles River Laboratories, Portal, Mass.) were reported as the 24-hour mean lethal dose (LD-50), as shown in Table 1. Mice were injected intraperitoneally with various amounts of the drug [usually at 1/4, 1/8 and 1/64 of the lethal LD-50] at 15 and 60 minutes before being given an intramuscular injection of 2xLD-50s [ca 6.7 mg/kg] of KCN. The number alive after 24 hours was recorded, and behavior testing on these mice was done at this time. As a control, 1000 mg/kg $Na_2S_2O_3$ and 100 mg/kg $NaNO_2$ were administered to ten mice 60 minutes prior to cyanide challenge.

The Comments column in Table 1 shows the effectiveness of the agents. A drug is considered PASSED if 4 out of 10 mice survived at any dose level, and INACTIVE if less than four survived. A drug is ACTIVE if it is still being screened.

The nitrite/thiosulfate pretreatment is very effective; usually 10 of 10 mice survive the 2 LD-50 of KCN. The basis of the treatment [7] is that this level of nitrate oxidizes about 30% of the Fe(II) hemoglobin into the high affinity Fe(III) methemoglobin state, which tightly binds cyanide in the blood. The thiosulfate serves as a substrate for rhodanese, which converts CN into SCN in the mitochondria. It has been shown [5] in guinea-pigs given 1000 mg/kg of sodium thiosulfate (either 0, 45 or 180 minutes before a long term infusion of NaCN), that the pigs were protected for about four hours before the onset of cyanide intoxication. Thus, the thiosulfate (LD-50 = 2.5 g/kg in rats) acts rapidly and protects for a long period of time, at this dose level. However, at levels of 100 and 500 mg/kg, the longer the time of pre-administration, the shorter was the threshold time before cyanide intoxication. The plasma half-life of thiosulfate is about 23 minutes in man. A dose of 1000 mg/kg thiosulfate which provided maximal protection in pigs, could be dangerous for humans. The opinion advanced [5] was that a usable prophylactic cyanide therapy does not yet exist, which is the basis for the present project.

It is important to understand the differences in the modes of action of thiosulfate and the scavenger drugs discussed below. The thiosulfate donates a mole of sulfane sulfur to the enzyme rhodanese, which changes CN into SCN, and the enzyme is then ready to transform more CN into SCN. If we administer 6.7 mg KCN [FW KCN = 65.12 mg/mM] /kg of mouse, to a 25 gm mouse, we have given 2.6 x 10(-03) mM CN to the mouse. With a dose of 1000 mg Na2S2O3.5H2O /kg [FW Na2S2O3.5H2O = 248.18 mg/mM] to a 0.025 kg mouse, we have give 0.1 mM sulfane S to the mouse. The ratio 0.1/[2.6 x 10(-03)] is 38/1. In other words, the thiosulfate, through the enzyme mechanism, is potentially capable of binding 37 times more cyanide than the amount actually administered. In addition, 100 mg/kg of NaNO2 was also give to the control mice. From the Charles River Labs, we learned that a 100 gm mouse contains 3.15 ml plasma and 5.85 ml whole blood. Also, there are 13.4 gms of hemoglobin (Hb) per 100 ml of whole blood. Thus, with a formula weight of 64,500 daltons for Hb and four moles of Fe/mole Hb, a 25 gm mouse contains 1.2 x 10(-02) moles of heme iron. If the 100 grams of sodium nitrite oxidizes ca 30% of the Hb to the Fe(III) methemoglobin, the mouse has 3.6 x 10(-03) mM Fe(III) to bind with the 2.6 x 10(-03) mM of cyanide administered. More than enough Met-Hb is present in the blood (1.5/1) to bind strongly with the total amount of cyanide present.

With the simple scavenger drugs, usually one mM of cyanide can be inactivated by one mM of drug, and a 37 fold excess of drug to administered cyanide is needed to be as effective as the amount of thiosulfate and nitrite given. Now, the amount of scavenger drug administered is limited by its toxicity, and for the best drugs which have LD-50s around 1000 mg/kg, the maximal dose is 1/4 LD-50, or 250 mg/kg, and assuming the FW of the drug is 250 mg/mM, we have administered 1 mM/kg or 2.5 x 10(-02) mM drug per 2.6 x 10(-03) mm CN, or at most a ten fold excess of drug/cyanide; more than three fold less than in the thiosulfate/ nitrite treatment. Most drugs will not have an LD-50 as great as 1000 mg/kg, and so the more toxic the agent, the less the dose given, and the lower the apparent effectiveness compared with the control.

Of the compounds submitted, Table 2 shows the theoretical number of moles of cyanide that a mole of drug should bind [THEORY CN:DRUG], compared with the amount experimentally found to be bound at pH 7.4 [EXP CN:DRUG], either using a cyanide specific electrode, or dedicated autoanalyzer. The results may be viewed as the <u>in vitro</u> basis for believing a particular drug should be effective <u>in vivo</u>, from a naive chemical perspective. For example sodium pyruvate had a low experimental binding ratio of 0.12 moles CN/mole of drug, perhaps indicating before-hand that it should be ineffective <u>in vivo</u>. Although it failed the present screen, literature work indicates that it has a limited <u>in vivo</u> scavenging abilities [9].

In Table 1 are listed the compounds studied, their LD-50s, and the ratio of the actual number of mM of drug given (ip) per mM of cyanide administered [D:CN]. Certain compound may be

TABLE 1. ANTI-CYANIDE DRUG RESULTS

ICD#	BOTTLE#	COMPOUND	LD50	D:CN[a]	COMMENTS
1092	BL 53684	Pd(NH3)4(NO3)2	126.50	0.9	INACTIVE
1093	BL 53693	Rh[III](NH3)6Cl3	62.40	0.4	INACTIVE
1094	BL 53675	Ru[IV]Cl6(NH4)2	500.00	6.0	INACTIVE
1095	BL 53666	K CROCONATE	500.00	6.0	INACTIVE
1096	BL 53657	TRANS Pd(DMSO)2Cl2	436.00	3.8	INACTIVE
1097	BL53648	4-PYRIDINE-CHO-N-OXIDE	1000.00	16.9	ACTIVE
1098	BL53639	4-QUINOLINE-CHO	158.50	2.1	INACTIVE
1099	BL 53620	GLYOXAL BISULFITE	300.00	7.3	INACTIVE
1100	BL53611	K-GOLD(III)Cl4		0.0	INACTIVE,
1101	BL 40276	GLYOXYLIC ACID,H2O	284.10	6.4	INACTIVE
1102	BL40267	ALPHA KETOMALONIC ACID	500.00	15.3	INACTIVE
1103	BL40258	FUMARIC ACID	1000.00	13.0	INACTIVE
1104	BL 40230	COBALT(II) CHLORIDE 6H2O	195.50	1.7	PASSED
1105	BL40221	CIS Pd(NH3)2Cl2	250.00	2.5	INACTIVE
1106	BL 40212	PYRIDOXAL-CHO	602.50	6.2	INACTIVE
1107	BL40203	CIS Pt(NH3)2Cl2	35.00	0.2	INACTIVE
1108	BL40196	TRANS Pt(NH3)2Cl2	251.70	1.7	INACTIVE
1109	BL40187	TRANS Pd(NH3)2Cl2			INACTIVE
1110	BL 40178	SODIUM PYRUVATE	1000.00	18.9	INACTIVE
1111	BL 40169	4-CARBOXYBENZALDEHYDE	500.00	13.9	INACTIVE
1112	BL 40150	5-CHO-FURAN-SO3	500.00	9.6	INACTIVE
1113	BL 40141	2-KETO-BUTYRIC ACID	1000.00	15.2	INACTIVE
1114	BL 40132	2,6-PYRIDINE-CHO	99.70	1.5	INACTIVE
1115	BL 40123	2-CARBOXYBENZALDEHYDE	776.40	10.8	PASSED
1116	BL40114	4-PYRIDINECARBOXYALDEHYDE	1250.00	19.5	INACTIVE
1117	BL40105	2-PYRIDINECARBOXALDEHYDE	315.00	0.0	INACTIVE

ICD#	BOTTLE#	COMPOUND	LD50	D:CN[a]	COMMENTS
1118	BL40098	3-PYRIDINECARBOXALDEHYDE		3.0	INACTIVE
1119	BL40089	PdCl4.2Na	315.00	2.3	INACTIVE
1128	BL 19146	Rh(III)-TPPS4/PORPHYRIN	245.00	1.4	INACTIVE
1129	BL 19137	Co(II)-PHTHALOCYANINE-SO3	325.00	1.7	INACTIVE
1130	BL 19128	Co(III)-TMPyP/PORPHYRIN	260.00	0.5	INACTIVE
1131	BL 19119	Cr(III)-TPPS4/PORPHYRIN	300.00	2.0	INACTIVE
1132	BL 19100	Co(III)-TPPS4/PORPHYRIN	650.00	1.1	INACTIVE
1133	BK 19093	Fe(III)-TMPyP/PORPHYRIN	100.00	0.2	INACTIVE
1137	BK 40995	Co(III)-TMPyP(3)/PORPHYRIN	320.00	0.5	INACTIVE
1138	BK 40977	Co(III)-PHTHALOCYANINE-SO3	450.00	0.8	INACTIVE
1139	BK 40968	Co(III)-TAP/PORPHYRIN	275.00	0.4	INACTIVE
1140	BK 40959	Co(III) HEMATOPORPHYRIN	500.00	3.0	INACTIVE
1141	BK 40940	Pd(II) HEMATOPORPHYRIN	500.00	3.0	INACTIVE
1142	BK 40931	Co(III)-TPPC4/PORPHYRIN	23.70	0.1	INACTIVE
1143	BK 40922	Co(III) PROTOPORPHYRIN	500.00	3.2	INACTIVE
1144	BK40913	C6H5-CH=(CN)CO2C2H5	500.00	7.2	INACTIVE
1145	BL 40904	C7H12=C(CN)CO2C2H5	44.90	0.5	INACTIVE
1146	BK40897	C6H10=C(CN)CO2C2H5	1000.00	0.0	ACTIVE
1147	BL 40888	O=C6H8=C(CN)CO2C2H5	500.00	10.1	INACTIVE
1148	BL 40879	C5H8=C(CN)COOH	10.00	0.1	INACTIVE
1149	BL 40860	C6H5-CH=C(CN)CO2H	500.00	12.0	INACTIVE
1150	BL 40851	m-NO2-C6H4-CH=C(CN)CO2H	500.00	9.6	INACTIVE
1171	BL 40249	NICKEL(II) CHLORIDE, 6H2O	299.00	2.6	PASSED

a. D:N is the experimental ratio of mM drug to cyanide administered

expected to bind more than 1mM of CN/mM of drug. For example, the experimental [D:CN] ratio is 2.6 for NiCl2, and since Ni(II) can bind four moles of CN, the effective D:CN ratio should be (2.6 x 4), or 10.4. Nevertheless, if the [D:CN] ratio is less than 1.0, probably too little drug has been administered to bind the total cyanide present. Thus, eleven out of forty-three compounds had ratios less than 1.0, and all eleven were INACTIVE. However, many compounds had [D:CN] ratios much greater than 1.0, and they were also INACTIVE. None of these ratios actually had that much predictive value. Of the 43 compounds run, only three PASSED the screen. A brief discussion of the compounds by class follows.

COMPOUNDS SUBMITTED BUT NOT RUN

The 2-pyridinecarboxaldehyde (ICD#1117) and 3-pyridinecarboxaldehyde (ICD# 1118) had noxious odors, and were not tested. The potassium tetrachlorogold(III) compound (ICN #1100) corroded the needles [the gold(III) was probably reduced by the Fe needles], and no further testing was attempted. The 4-pyridinecarboxaldehyde N-oxide (ICD# 1097) and the ethyl-alpha-cyclohexylidine-alpha-cyanoacetate, (ICN# 1146) had LD-50s around 1000 mg/kg, but were in insufficient amounts for further work. We submitted more of each sample, and they are presently on the ACTIVE list. A silver(II) porphyrin (BK 40986) was misplaced, and has not been run. The trans Pd(NH3)2Cl2 (ICD# 1109) was depleted, and it appeared from the work done to be INACTIVE.

METALLOPORPHYRINS AND SULFONATED PHTHALOCYANINES

In Table 1, the phthalocyanine-SO3 indicates tetrasulfonated phthalocyanines, while TPPS4 is tetrakis(4-sulfonatophenyl)-porphyrin, TMPyP is tetrakis(N-methyl-4-pyridyl)porphyrin and TMPyP(3) is its 3-pyridyl isomer. None of the water soluble phthalocyanines [Co(II), Co(III)] passed the screen, nor did any of the positively or negatively charged porphyrins [(Co(III), Fe(III), Cr(III), Rh(III), Pd(II)]. The rhodium compound was the best of the lot, and has been suggested as a scavenger agent before [10], as have several of the Co(III) porphyrins [11]. Both Rh(III) and Co(III) porphyrins have high <u>in vitro</u> affinity for cyanide in their oxidized states, and the Rh(III) adduct is most difficult to reduce. We discuss in a later section pulsed-radiolysis work on the reduction of the cyano-Co(III) porphyrins, which lose their cyanides when reduced to Co(I). The porphyrins are about a factor of four times more toxic than hydroxo-cobalamin (2 g/kg [12]), and this may be due to the COOH, SO3-, positive pyridinium or N(CH3)3 functions present on the porphyrin periphery. The B-12 has mainly -CO-NH2 groups around the central core.

ALKYLIDENES

TABLE 2. THEORETICAL AND EXPERIMENTAL CYANIDE / DRUG BINDING RATIOS

COMPOUND	THEORY CN:DRUG[a]	EXP CN:DRUG[b]
Pd(NH3)4(NO3)2	4.0	4.00
Rh[III](NH3)6Cl3	6.0	0.09
Ru[IV]Cl6(NH4)2	6.0	1.00
K CROCONATE	1.0	0.15
trans-Pd(DMSO)2Cl2	4.0	4.00
4-PYRIDINE-CHO-N-OXIDE	1.0	0.82
4-QUINOLINE-CHO	1.0	0.60
GLYOXAL BISULFITE	2.0	0.70
K-GOLD(III)Cl4	4.0	3.00
GLYOXYLIC ACID,H2O	1.0	1.00
ALPHA KETOMALONIC ACID	1.0	0.17
FUMARIC ACID	1.0	0.10
Co(II) CHLORIDE 6H2O	5.0	5.00
CIS Pd(NH3)2Cl2	4.0	4.00
PYRIDOXAL-CHO	1.0	0.10
CIS Pt(NH3)2Cl2	4.0	2.00
TRANS Pt(NH3)2Cl2	4.0	2.00
TRANS Pd(NH3)2Cl2	4.0	4.00
SODIUM PYRUVATE	1.0	0.12
4-CARBOXYBENZALDEHYDE	1.0	0.15
5-CHO-FURAN-SO3	1.0	0.08
2-KETO-BUTYRIC ACID	1.0	0.43
2,6-PYRIDINE-CHO	2.0	2.00
2-CARBOXYBENZALDEHYDE	1.0	0.18
4-PYRIDINECARBOXYALDEHYDE	1.0	0.50
2-PYRIDINECARBOXALDEHYDE	1.0	0.80

COMPOUND	THEORY CN:DRUG[a]	EXP CN:DRUG[b]
3-PYRIDINECARBOXALDEHYDE	1.0	0.50
PdCl4.2Na	4.0	4.00
Rh(III)-TPPS4/PORPHYRIN	2.0	2.00
Co(II)-PHTHALOCYANINE-SO3	2.0	2.00
Co(III)-TMPyP/PORPHYRIN	2.0	2.00
Cr(III)-TPPS4/PORPHYRIN	2.0	2.00
Co(III)-TPPS4/PORPHYRIN	2.0	2.00
Fe(III)-TMPyP/PORPHYRIN	2.0	2.00
Co(III)-TMPyP(3)/PORPHYRIN	2.0	2.00
Co(III)-PHTHALOCYANINE-SO3	2.0	2.00
Co(III)-TAP/PORPHYRIN	2.0	2.00
Co(III) HEMATOPORPHYRIN	2.0	2.00
Pd(II) HEMATOPORPHYRIN	2.0	2.00
Co(III)-TPPC4/PORPHYRIN	2.0	2.00
Co(III) PROTOPORPHYRIN	2.0	2.00
C6H5-CH=(CN)CO2C2H5	1.0	0.10
C7H12=C(CN)CO2C2H5	1.0	0.10
C6H10=C(CN)CO2C2H5	1.0	0.10
O=C6H8=C(CN)CO2C2H5	1.0	0.10
C5H8=C(CN)COOH	1.0	0.10
C6H5-CH=C(CN)CO2H	1.0	0.10
m-NO2-C6H4-CH=C(CN)CO2H	1.0	0.10
NICKEL(II) CHLORIDE, 6H2O	4.0	4.00

a. THEORY CN:DRUG is the theoretical amount of cyanide the drug can bind

b. EXP CN:DRUG is the actual amount of cyanide found to be bound by the drug in vitro at pH 7.4, using a cyanide electrode

ALKYLIDENES

Alkylidenes are organic molecules containing a double bond, which has an attached electron withdrawing function: a -CN group in these examples. The withdrawing effect of the cyanide activates the double bond to cyanide addition. If an electron withdrawing group is not present, cyanide is difficult to add. Even with an electron withdrawing function, we find the cyanide addition reactions are slow at room temperature as judged by our cyanide electrode and spectrophotometric work, for reasonable millimolar cyanide concentrations. None of the alkylidenes passed the screen. The saturated C6H10-CH=(CN)CO2C2H5 (ICD # 1146) had an LD-50 of 1000 mg/kg, and is still under investigation. These compounds add cyanide so slowly at room temperature, that whatever effects are found may be due to other factors, such as active site blocking. It is interesting that fumaric acid, which showed basically no cyanide uptake in solution (having a non-activated double bond), saved no mice under any dose level conditions.

CYANOHYDRIN FORMERS

A variety of aldehydes and ketones were submitted. Although sodium pyruvate protects mice to a limited extent [9] against cyanide, it failed this screen. as did many compounds which were effective <u>in vitro</u>. Thus, the 2,6-pyridinecarboxaldehyde bound two moles of cyanide rapidly in solution, but it had a low LD-50 (99.7 mg/kg), and larger amounts could not be administered. The only compound to have PASSED the screen in this category was 2-carboxybenzaldehyde (ICD# 1115), with an LD-50 of 776.4 mg/kg body weight, and it bound only 0.18 moles CN per mole of drug at pH 7.4. The amount of drug, time of pre-administration and number of mice surviving (out of ten tested) are given below.

	194mg/kg	48.5	12.0	3.0mg/kg
60 min	1	2	1	0
15 min	3	4	2	0

The carbonyl group of chloral, 2,2,2-trichloroacetaldehyde is hydrated in solution to chloral hydrate [CCl3CH(OH)2], and can form hemiacetals of the formula CCl3CH(OH)OR, which generate chloral hydrate <u>in vivo</u>. Chloral hydrate is rapidly reduced to trichloroethanol(CCl3CH2OH) within a few minutes of administration by the alcohol dehydrogenase in the liver [13], and a small fraction is oxidized to trichloroacetic acid in the liver and kidneys. This may be the fate of many of the aldehydes

screened, and thus account for their lack of cyanide uptake behavior. The 2-carboxybenzaldehyde has a tautomeric form which may inhibit such rapid oxidation.

CLASSIC METAL ION COMPLEXES

We found several gold(III) compounds, AuBr3 and KAuCl4 to bind three moles of cyanide in solution, but the Au(III) was reduced by the Fe needles in the screen, and the tests were discontinued. Palladium(II) complexes rapidly scavenged four moles of cyanide in vitro. However, none of the Pd(II) [nor Pt(II)] complexes were found effective. The best complexes were the hexaquo salts of nickel(II) and cobalt(II) chloride, both of which PASSED the screen.

For NiCl2.6H2O, which rapidly binds four moles of cyanide at pH 7.4, the LD-50 was 299.00 mg/kg body weight.

	75 mg/kg	18.7	4.7	1.2mg/kg
60 min	10	6	0	1
15 min	10	9	3	0

For CoCl2.6H2O, which rapidly reacts with five moles of cyanide at pH 7.4, and the LD-50 is 195.50 mg/kg body weight.

	48.9mg/kg	12.2	3.0	0.75mg/kg
60 min	9	2	0	0
15 min	9	0	0	0

Cobalt(II) salts are known cyanide antidotes, especially dicobalt-EDTA [7]. The Ni(II) results are new in this field, and the data indicates that Ni(II) is somewhat more effective than Co(II). It is noted that cobalt(II) forms a strong reducing agent, the pentacyanocobaltate ion upon binding five moles of cyanide, and may be rapidly oxidized to the very stable and substitution inert aquo-Co(III)-pentacyano derivative. The tetracyanonickelate is substitution labile, with a small [14] dissociation constant [ca 10(-30)], and remains in the in the divalent state.

CURRENT WORK

As noted earlier, we submitted hydroxocobalamin and a dicobalt-EDTA to find how effective these substances would be in the present screen. Since aquo Ni(II) was good, we added a mole of EDTA forming Ni(EDTA)2-, and found it to complex 0.14 moles of

CN, compared to 4.0 for aquo Ni(II). Thus, at pH 7.4, the EDTA makes the Ni(II) less available for cyanide uptake. We found that aquo Co(II) binds five moles of cyanide in solution, whereas the Co(EDTA)2- binds only 0.1 mole. Addition of another mole of Co(II) forms Co(CoEDTA), and this species bound 6.0 moles of cyanide per mole of EDTA, or three moles per mole of cobalt. Thus, it is the extra cobalt that leads to cyanide binding, and it is this same extra cobalt that perhaps leads to later cardiac problems. The EDTA may reduce the toxicity of the free metal ions, but at this pH, it also renders them less effective cyanide agents.

The cobalt(III) porphyrins were excellent cyanide binders in solution, and could form dicyano adducts. The pH maximum for cyanide uptake was in the physiologic range, and one of the cyanides seemed to be irreversibly bound to the cobalt atom [11]. Nevertheless, none of these species PASSED. It was possible that the di-cyano complexes were reduced to lower Co-porphyrin oxidation states <u>in vivo</u>, and thus lost a substantial amount of binding effectiveness. To test this hypothesis, we did reductive pulsed-radiolysis and electrochemical studies on several of the cobalt porphyrins. For all compounds tested, in the absence of cyanide, the Co(III)-porphyrin could be reduced in stages to Co(II) and then the Co(I) states, and finally two electrons were added on the ligand itself. For CoTPPS [tetra(4-sulfonatophenyl)porphyrin] in the (CN)2CoIII-TPPS complex, the Co(II) and Co(I) forms could again be produced, where spectra shifts indicated that the CoII-TPPS was still bound by cyanide, while the Co(I) state was cyanide free. A different pattern was noted for (CN)2CoIIITMPyP [tetra(N-methyl-4-pyridyl)porphyrin]. Upon reduction, one electron was added not to the metal but to the porphyrin ring forming the mono-anion-(CN)2-Co(III)-porphyrin, and two of these species disproportionate into the initial dicyano-Co(III)TMPyP and the dicyano-Co(III) porphyrin di-anion, having two electrons added to the porphyrin ring. Thus, depending on the porphyrin, different modes of reduction in the presence of cyanide are observed. In another set of experiments, Co(II)-TMPyP was produced by radiolysis, and when cyanide was added, the dicyano-Co(III)-radical anion of TMPyP was produced, indicating that the electron from Co(II) moved from the metal center into the ring under the influence of cyanide. This species then underwent disproportionation as noted above. Using cyclic voltammetric techniques, the Co(II) and Co(I) states of all porphyrins were produced in the absence of cyanide. For CoTPPS, the reduction wave was shifted to more negative potentials in the presence of cyanide, indicating that cyanide stabilized the oxidized state, and the position of the oxidation wave indicated some cyanide affinity for Co(II)-TPPS in the reduced state. All cyanide binding tendencies were lost in the Co(I) product. On the other hand, the (CN)2-CoIII-TMPyP was reduced at the electrode in a single two-electron step forming the ring reduced phlorin. In contrast, hydroxocobalamin [15] is reduced in an apparent two electron step at the electrode, where the product is Co(I). This (CN)2-CoIII B12 is more difficult to reduce than porphyrins, and while Co(II) B-12 only weakly binds one mole of cyanide, the

Co(I) form has no cyanide affinity. Thus, both radiolysis and electrochemical work indicated some stability for cyano complexes in the Co(II)-porphyrin state, but none for Co(I). A publication is planned [16].

In order to create molecules which would release a cyanide scavenger metal only in the presence of the strong nucleophile cyanide, we studied a number of macrocyclic complexes, in particular N-methyl-porphyrin complexes of Ni(II). The N-methyl group replaces one of the central protons in the porphyrin central cavity, and upon complexation of Ni(II), this metal sits above the plane of the porphyrin molecule, forced above by the N-methyl group. In non-N-alkylated Ni(II) porphyrins, the Ni(II) is in the porphyrin plane, and has no affinity for cyanide. The out-of-plane Ni(II) in the N-methyl porphyrins rapidly binds with four cyanide ligands, and is removed from the porphyrin as the presumably non-toxic Ni(CN)42-, a reaction which stops when the cyanide in solution is depleted. Thus, in the absence of cyanide, the nickel is strongly complexed and unavailable, whereas in the presence of cyanide, a potent scavenger results. The situation is more complicated with Co(II) N-alkyl porphyrins, which seem to retain the metal (which might be oxidized) upon cyanide addition. Further work is planned along these lines.

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