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Cyanide Antidotes for Mass Casualties: Water-Soluble Salts of the Dithiane (Sulfanegen) from 3-Mercaptopyruvate for Intramuscular Administration

Steven E. Patterson[*,†], **Alexandre R. Monteil**[†], **Jonathan F. Cohen**[†], **Daune L. Crankshaw**[†,‡], **Robert Vince**[†], and **Herbert T. Nagasawa**[†]

[†]Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455

[‡]Medical Research Laboratories, DVA Medical Center, Minneapolis, MN 55417

Abstract

Current cyanide antidotes are administered by IV infusion which is suboptimal for mass casualties. Therefore, in a cyanide disaster intramuscular (IM) injectable antidotes would be more appropriate. We report the discovery of the highly water-soluble sulfanegen triethanolamine as a promising lead for development as an IM injectable cyanide antidote.

Introduction

Improving the efficiency of cyanide detoxifying enzyme pathways by supplying substrates in prodrug form should be a practical approach for the design of an antidote. There are two mammalian enzymes that sequester cyanide as thiocyanate, (a urinary detoxification product of cyanide) viz., rhodanase (thiosulfate/cyanide sulfurtransferase, EC 2.8.1.1) and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2). Of these, 3-MST is preferred, because of its ubiquitous presence in most organs including the central nervous system and its intracellular distribution in the cytosol as well as in the mitochondria.[1-4] However, the reported instability in blood of 3-mercaptopyruvate (3-MP),[5] the transamination product of L-cysteine and the endogenous substrate of 3-MST for sulfur transfer, renders this substrate/enzyme system difficult to exploit for use in cyanide antidote therapy.

Therefore, by adopting prodrug principles,[6] a series of highly effective prodrugs and double prodrugs of 3-MP (Figure 1) were prepared as potential cyanide antidotes.[7] Some of these were shown to be not only orally bioavailable with rapid onset of action, but could also be expected to provide prophylaxis against imminent cyanide exposure due to their delayed action, the latter antidotes requiring sequential bioactivation steps to release 3-MP.[7] Orally effective anti-cyanide agents, previously unknown, could be extremely useful for first responders in the event of a cyanide disaster precipitated by a major chemical accident or a

[*]**Corresponding Author**: Tel: 612-625-7962, FAX: 612-625-8154, patte219@umn.edu..

Supporting Information.
Experimental details for the preparation of **6** and **7a-f**, experimental data covering the NMR studies of **2,** and the evaluation of **2** and **7e** in the sublethal murine cyanide model. X-ray crystallographic data for compounds **7d** and **7e.** These are available free of charge via the Internet at http://pubs.acs.org

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terrorist-caused incident. However, such oral antidotes could only be given to ambulatory subjects exposed to cyanide, leaving the comatose victims the only option of intravenous antidote therapy, a relatively slow and elaborate procedure requiring highly trained paramedical personnel. The consequence of such a scenario would be to leave large numbers of cyanide-exposed victims untreated in any rescue attempt.

Accordingly, it was incumbent on us to devise much more rapid delivery methods for the treatment of mass casualties, perhaps also aided by deployment of rapid action mechanical devices. Although sublingual and trans-dermal delivery are possibilities, the absorption rates of the antidotes via such routes were deemed to be much too slow for the treatment of a rapid acting poison like cyanide. Thus, the viable choices remaining are to administer the antidote by the intramuscular (IM), intraosseous (IO), or intranasal/intratracheal routes. This report focuses on the first.

The IM mode for cyanide antidote administration poses a technical challenge in that (a) large doses must be administered within a short time for maximal efficacy; (b) inflammatory responses must be minimal at the IM injection site; hence, biocompatibility is of prime consideration; and (c) the antidote must be highly water-soluble as a consequence of (a). If an anti-cyanide agent fulfilling the above criteria can be prepared and developed, treatment of mass casualty cyanide victims could become reality, especially if mechanical injection devices presently available commercially, or adapted for this use, can be implemented.

We have demonstrated that the sodium salt of the dimeric, dithiane form of 3-mercaptopyruvate (sulfanegen sodium, compound **2**, Figure 2), is a highly potent antidote in our sublethal mouse and lethal piglet models by IP and IV injection, respectively.[7, 8] However, it became clear that this salt could not fulfill the above role, since its aqueous solubility was not greater than 128 mg/mL (0.35 M), and dose calculations suggested that a minimum water solubility of 1.05 M was required for antidotal efficacy by the IM route for humans, based on a 60 kg human with a maximum injectable volume of 5 mL.[9]

Cognizant of this requirement for biological compatibility in addition to high water solubility, we embarked on a synthetic program to produce other salt forms of sulfanegen different from **2**, preferably with biocompatible organic amines, and tested them individually in our mouse model for assessing antidotal efficacy, using sub-lethal doses of cyanide and comparing the righting reflex recovery times of the antidote-treated vs. untreated mice.[10] In this model, the antidotal efficacy is measured by reduction in time required for the mouse to recover neuromuscular coordination after a toxic, but sublethal dose of cyanide.

Results

In order to understand the chemistry and dissociation propensities of the salt forms of the dimeric, 3-mercaptopyruvate dithianes, it was necessary to study the stability/instability at physiological pH and temperature of the prototype sodium salt, **2**. Lack of a chromophore in dithianes required NMR methods for such studies, and this was aided by the observation that the methylene groups in the dithiane exhibited an ABX pattern (dd) centered at 3.67 and 2.66 ppm,[7] whereas in the partially ring-opened α-keto acid form **3**, these methylene protons exchanged with solvent D_2O, via the enol **4 (**Fig. 2), and their proton intensities gradually diminished over time, this rate of reduction being measurable quantitatively by integration vs. t-butyl alcohol as an internal standard.

Such studies in D_2O/DCl or D_2O/deuterated phosphate buffer demonstrated that, whereas **2** was essentially stable to dissociation (no deuterium exchange) at pH 1.4 (pD 1.0) over the

22 hour period observed, it readily underwent ring-opening at the physiologic pH of 7.4 (pD 7.0) with a half-life of 2.0 hours (Supplementary Material).

The above data allowed the facile and quantitative preparation of the stable acid **6**[11] of the dithiane by passing **2** through a cation exchange column in the acid form (Scheme 1). Salts of **6** with biologically compatible organic amines could then be prepared simply by adding a stoichiometric amount of the counter base and removal of the solvent by lyophilization or other means, Table 1 summarizes the water-solubility (shake flask method)[12] properties of these sulfanegen salts.

We expected the most biologically compatible salt forms to be those prepared from tromethamine [tris-(hydroxymethyl)aminomethane; Tris] or better, from D-glucosamine, a widely consumed dietary supplement, viz., compounds **7f** and **7b** (Scheme 1). However, their water solubilities on a molar basis were only slightly greater than the sodium salt **2** (Table 1). Based on aqueous solubilities alone, the most promising salts for our purposes were **7a, 7c, 7d** and **7e** prepared by treatment of **6** with meglumine, ethanolamine, diethanolamine (DEA), and triethanolamine (TEA), respectively. All these salts had solubilities in H2O of greater than 1 M (Table 1). Salt **7a,** although extremely soluble, was a hygroscopic glass that proved difficult to handle and was, therefore, thought to be unsuitable for further development. Salt **7c** had solubility only marginally greater than our calculated minimum. Thus, we focused on salts **7d** and **7e**. TEA has been reported to show mild toxicity with chronic oral administration, is slightly better tolerated than DEA[13] and IM administration of TEA alone to mice at high doses showed mild, temporary symptoms of discomfort. Additionally, due to the reported use of TEA salts in the formulation of analgesics,[14-17] the highly water soluble TEA salt **7e** was selected over **7d** as the salt of choice for IM administrations.

Using our standard dose-response test system[10] as a measure of how well the salts were tolerated in mice, we found that TEA salt **7e** was indeed as well tolerated as the sodium salt **2**, and both were better tolerated than the other amine salts examined.

Salt **7e** was then tested in the above system[10] where antidotal efficacy is measured by reduction in the time required for recovery from a sublethal dose of cyanide i.e. the shorter time indicates greater antidotal efficacy. When administered IM 5, 10, 20, 30 and 40 minutes post cyanide (IM injections bilaterally into each thigh muscle), the TEA salt **7e,** even at lower doses**,** demonstrated superior efficacy relative to the sodium salt **2** at the limit (o.40 M) of the latter's solubility (Table 2). This experimental protocol was designed to assess the antidotal efficacies of **7e** relative to the possible arrival times of the "first responders" following a major cyanide disaster. It is clear that the TEA salt **7e** adequately fulfilled our expectations.

In summary, we have identified a highly water-soluble sulfanegen salt, viz., sulfanegen TEA (**7e**), which should be amenable for development as an IM injectable antidote suitable for treatment of cyanide victims in a mass casualty setting. Further development, including efficacy in lethal cyanide animal models, will be reported at a later date.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

3-MP	3-mercaptopyruvate
3-MST	3-mercaptopyruvate sulfurtransferase
DEA	diethanolamine
IM	intramuscular
IO	intraosseous
SE	standard error
TEA	triethanolamine

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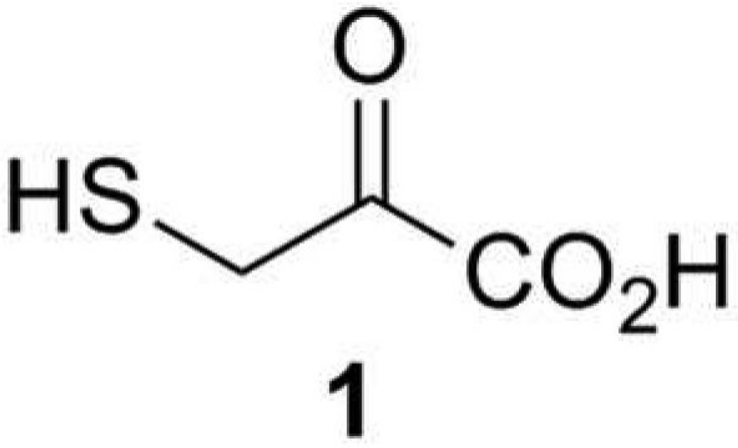


Figure 1.
3-Mercaptopyruvic acid (3-MP), the endogenous substrate for 3-MST

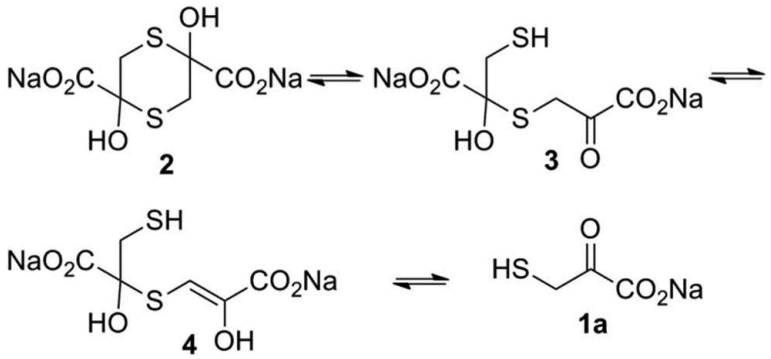
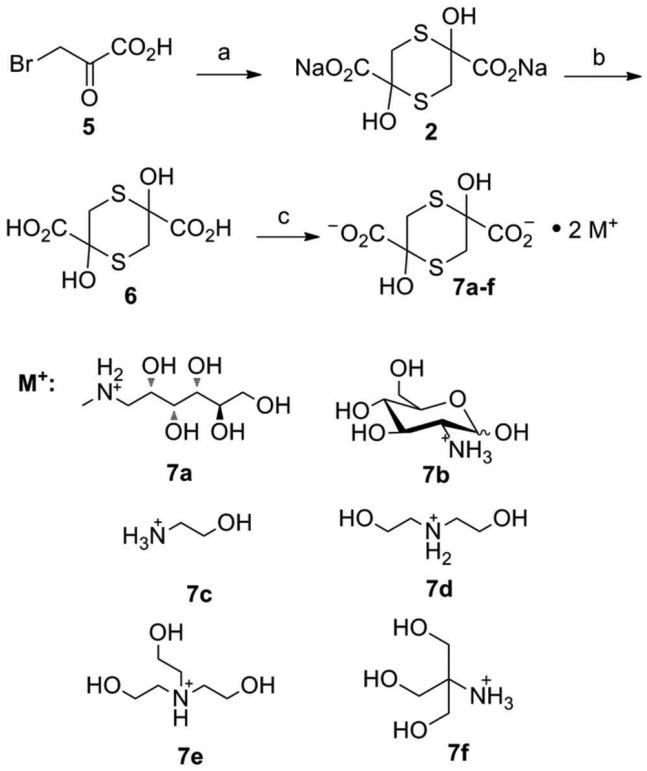


Figure 2.
Chemical equilibria of dithiane **2**



Scheme 1. Synthesis of sulfanegen salts 7a-f

[a]Reagents and conditions: (a) 2 molar equivalents NaHS, ethanol, 0 °C; (b) Dowex-50WX8, H^+ form, 7 equivalents; (c) 2 equivalents of a biocompatible amine (M).

Table 1

Solubility of sulfanegen salts

Compound	*Solubility (M @ 20 °C)*	*MP (°C)*
2	0.35	132-134
6	0.19	148-150
7a	1.95	119-120 (dec)
7b	0.49	126-128
7c	1.05	73-75
7d	2.25	104-105
7e	1.58	122-123
7f	0.48	125-127

Table 2

Recovery time (minutes ± SE) in the sublethal murine cyanide model for antidotes administered post cyanide at the time indicated

Treatment (dose)*	5 min	10 min	20 min	30 min	40 min
Saline	68.3 ± 3.2	68.6 ± 1.3	74.5 ± 1.9	68.0 ± 2.0	69.0 ± 2.1
7e (0.018)	26.8 ± 1.1	34.0 ± 1.7	41.8 ± 1.0	55.5 ± 1.7	64.0 ± 1.7
7e (0.073)	16.8 ± 0.3	24.0 ± 0.6	36.5 ± 2.0	51.3 ± 1.3	52.0 ± 3.2
7e (0.29)	14.5 ± 0.3	23.7 ± 0.3	34.3 ± 0.5	47.0 ± 1.5	44.8 ± 0.5
7e (0.73)	10.8 ± 0.8	19.4 ± 0.8	31.3 ± 2.9	42.0 ± 0.6	43.0 ± 0.7
2 (0.73)	19.7 ± 0.3	31.0 ± 0.5	44.8 ± 1.2	49.0 ± 1.2	58.7 ± 0.5

*dose of antidote in moles/kg body weight. Dose of NaCN 4.8 mg/kg IP