Prevention and Treatment of Vesication and Poisoning Caused by Arsenicals

Annual Summary Report

September 15, 1982

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PREVENTION AND TREATMENT OF VESICATION AND POISONING CAUSED BY ARSENICALS

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PART I ANTI-LEWISITE ACTIVITY AND STABILITY OF MESO-DIMERCAPTOSUCCINIC ACID AND 2,3-DIMERCAPTO-1-PROPANESULFONIC ACID

Meso-dimercaptosuccinic acid (DMSA) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) are analogous in chemical structure to dimercaprol (BAL, British Anti-Lewisite). Dimercaprol was among the first therapeutically useful metal chelating agents and was developed originally as an anti-Lewisite agent. Either DMSA or DMPS protects rabbits from the lethal
systemic action of dichloro(2-chlorovinyl)arsine (29.7 μmols/kg, also known as lewisite). The analogs are active in this respect when given either sc or po. The stability of each of the three dimercapto compounds in distilled H₂O, pH 7.0 at 24°, has been examined for seven days. DMSA retained 82% of its mercapto groups, but no titratable mercapto groups remained in the DMPS or BAL solutions. At pH 5.0, however, there was no striking difference in the stability of the three dimercapto compounds (78-87%) over a seven day period. DMSA and DMPS warrant further investigation as water soluble metal binding agents in both in vivo and in vitro experiments.

PART II - Optical Isomers of 2,3-Dimercapto-1-propanesulfonate:
Antidotal Activity, in vitro and in vivo, against Sodium Arsenite.

DMPS (2,3-dimercapto-1-propane sulfonate, Na salt) is an important water soluble analog of dimercaprol. All investigations of this antidote for heavy metal intoxication have dealt only with the racemic mixture. In the present report, the optical isomers of DMPS have been separated and the arsenic-antidote activity of the levorotatory (-) isomer, the dextrorotatory (+) isomer and the racemic mixture of DMPS have been investigated in vivo and in vitro. The individual optical isomers and the racemic mixture of DMPS are effective equally, in vitro, in preventing the inhibition by sodium arsenite of the activity of mouse kidney pyruvate dehydrogenase complex (PDH). In addition, when PDH is inhibited, in vitro, by sodium arsenite, any of the three DMPS preparations will reverse the inhibition equally well. The in vitro evidence suggests that two molecules of DMPS are required to prevent the effects of one molecule of sodium arsenite. Neither the LD50s nor the ED50s of each of the three forms of DMPS differ significantly when measured i.p. in mice. In addition, there is no striking difference between the effectiveness of the levorotatory or dextrorotatory DMPS when given orally to mice challenged with sodium arsenite. Thus, the use of the individual optical isomers of DMPS does not appear to have any advantage over the racemic mixture as an arsenic antidote under these conditions.

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Distribution Statement A is correct for this report.
Per Mrs. Virginia Miller, AMRDC/SGRD-FMI-S
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SUMMARY

Purpose: to find ways to prevent vesication and poisoning caused by arsenicals including lewisite.

Major methods up to this stage: Protection of rabbits against the lethal effects of lewisite; protection of mice against lethal effects of sodium arsenite; and prevention and reversal of PDE inhibition as in vitro screen.

Summary of results: PART I ANTI-LEWISITE ACTIVITY AND STABILITY OF MESO-DIMERCAPTOSUCCINIC ACID AND 2,3-DIMERCAPTO-1-PROPANESULFONIC ACID

-Meso-dimercaptosuccinic acid (DMSA) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) are analogous in chemical structure to dimercaprol (BAL, British Anti-Lewisite). Dimercaprol was among the first therapeutically useful metal chelating agents and was developed originally as an anti-lewisite agent. Either DMSA or DMPS protects rabbits from the lethal systemic action of dichloro(2-chlorovinyl)arsine (29.7μmols/kg, also known as lewisite). The analogs are active in this respect when given either sc or po. The stability of each of the three dimercapto compounds in distilled H₂O, pH 7.0 at 24°, has been examined for seven days. DMSA retained 82% of its mercapto groups, but no titratable mercapto groups remained in the DMPS or BAL solutions. At pH 5.0, however, there was no striking difference in the stability of the three dimercapto compounds (78-87%) over a seven day period. DMSA and DMPS warrant further investigation as water soluble metal binding agents in both in vivo and in vitro experiments.

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inexpensive, very accurate and uses fewer animals than the in vivo screen. The in vivo screen is now our second screen.

Conclusion: It would appear that DMPS, DMSA and other dimercapto compounds warrant further experimental studies and eventually clinical trials for the treatment of intoxication by arsenic, especially against lewisite gas. These agents have been used in human therapy in the Soviet Union and China. Soviet investigators and West German investigators have recommended that it replace BAL for treatment of heavy metal poisoning.
FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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PART I ANTI-LEWISITE ACTIVITY AND STABILITY OF MESO-DIMERCAPTOSUCCINIC ACID AND 2,3-DIMERCAPTO-1-PROPANESULFONIC ACID

Summary

Meso-dimercaptosuccinic acid (DMSA) and the sodium salt of 2,3- dimercapto-1-propanesulfonic acid (DMPS) are analogous in chemical structure to dimercaprol (BAL, British Anti-Lewisite). Dimercaprol was among the first therapeutically useful metal chelating agents and was developed originally as an anti-lewisite agent. Either DMSA or DMPS protects rabbits from the lethal systemic action of dichloro(2-chlorovinyl)arsine (29.7 μmols/kg, also known as lewisite. The analogs are active in this respect when given either sc or po. The stability of each of the three dimercapto compounds in distilled H2O, pH 7.0 at 24°, has been examined for seven days. DMSA retained 82% of its mercapto groups, but no titratable mercapto groups remained in the DMPS or BAL solutions. At pH 5.0, however, there was no striking difference in the stability of the three dimercapto compounds (78-87%) over a seven day period. DMSA and DMPS warrant further investigation as water soluble metal binding agents in both in vivo and in vitro experiments.

British Anti-Lewisite (BAL, dimercaprol) was developed in the 1940's as an antidote to dichloro-(2-chlorovinyl)-arsine, commonly called lewisite (1,2). The lethal action of lewisite is believed to be the result of its combining with one or more sulfhydryl groups and thus inactivating essential sulfhydryl-containing enzymes (3). It is the arsenic in the lewisite molecule that reacts with sulfhydryl moieties.

At the time of its introduction into clinical medicine, BAL was considered by many to be the long sought, universal antidote for heavy metal poisoning. In subsequent years, however, less toxic and more specific metal binding agents have been sought and investigated. Some have met the criteria
and standards necessary for clinical use. Others have not. For example, BAL glucoside was introduced (4) as a result of a search for water soluble and less toxic analogs of BAL. Although it was found to be less toxic than BAL for iv use, (probably because of its low lipid solubility), it did not become established as a clinical agent because it is unstable chemically. Other compounds, which are less analogous in chemical structure, have replaced BAL for some of its more specific therapeutic uses. For example, D-penicillamine is used to mobilize and increase the excretion of copper in patients with Wilson’s Disease (5). Its N-acetyl derivative is effective as a mercury antidote (6,7). BAL has remained, however, the drug of choice in the U.S. for the treatment of arsenic poisoning.

Meso-dimercaptosuccinic acid (DMSA) (8) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) (9) are promising replacements for BAL. These compounds are very similar in chemical structure to BAL and are sometimes referred to as water soluble and/or orally-effective analogs of BAL. To our knowledge, however, the anti-lewisite activity of these two important chemical analogs has not been determined. Neither are any published data available concerning the stability of aqueous solutions of these dimercapto compounds. Evidence for the anti-lewisite activity and stability of DMPS and DMSA are presented in this paper.

Materials and Methods

Male New Zealand white stock rabbits weighing 2.5-3.5 kg were purchased from Dutchland Laboratories Inc., Denver, PA and Davidson Mill Farm, Jonesbury, NJ and caged individually. Food (Purina Rabbit Chow Brand 5322) and water were available ad libitum except in the case of those animals who received therapy orally. Animals receiving therapy po were fasted from 16 hrs prior to the first administration to 1 hr after the last administration.
on day one. On days two and three, animals were fasted from 1 hr prior to
the morning administration to 1 hr after the evening administration,
approximately 7 hours.

When dithiol therapy was given sc, the animals were anesthetized fifteen
minutes before lewisite administration by administering im 0.50 ml of
anesthetic solution per kg. The animals were anesthetized to reduce the pain
expected to be caused by lewisite. Subsequently, it was observed that
neither pain nor discomfort was apparent. Thus, anesthesia was not used in
the experiments when dithiols were given po. The anesthetic solution was
prepared by mixing 5 parts Ketamine HCl (100 mg/ml) and 1 part of Xylazine
(100 mg/ml).

A 5 ml Gilson Pipetman was used to give the dithiols by mouth. The
rabbit was placed in a short restraining box. The box was placed on its end
so that the rabbit was in a vertical position with its head at the top. The
Pipetman was filled with the desired volume of the drug solution. The
plastic tip was gently inserted between the lips at one corner of the mouth
and the liquid delivered slowly into the back of the rabbit's mouth. This
method did not appear to cause any trauma or injury. It was easier and
faster to perform than the use of polyethylene stomach tubes.

NaDMPS was a gift of Heyl and Co., Berlin. Since each molecule has a
molecule of H₂O associated with it, a molecular weight of 228.2 was used in
calculations. DMSA was a gift of Johnson and Johnson, Skillman, N.J. Both
compounds were pharmaceutical grade purity. DMPS and DMSA were titrated with
iodine in order to measure purity and mercapto content. By this criterion,
each preparation was judged to be greater than 99% pure. The compounds when
given by mouth were dissolved in water. In order to dissolve DMSA, the
aqueous suspension was adjusted to pH 5.5 with NaOH. When given sc, the
solutions were prepared the same way except that the compounds were dissolved in 0.9% NaCl-5% NaHCO₃. Unless otherwise stated, the concentrations of DMPS or DMSA were such that the rabbit received 1.0 ml of solution per kg of body weight, per administration. Dimercaprol Injection, USP (BAL in Oil Ampules) was a gift of Hynson, Wescott & Dunning, Baltimore, MD.

Lewisite was 97-99.6% pure as judged by NMR-spectroscopy as well as by iodine titration. Analysis by the former method also indicated that the forms of lewisite that were present were trans (97.7%), cis (1.7%) and dimers (0.5%). Lewisite is a hazardous material with which to work since it is a potent vesicant. All handling of lewisite was done in an extremely well-vented chemical exhaust hood. Safety glasses and thick neoprene gloves were worn.

The stability of DMPS, DMSA or BAL was determined using iodometric titration. To 2.50 ml of a 0.10 M dimercapto solution, 10 drops of starch indicator solution were added. The solution was titrated using 0.025 N iodine solution until the blue color appeared and persisted for at least 10 sec.

Results

Anti-Lewisite Activity

The data of Table 1 clearly show that both DMSA and DMPS have anti-lewisite activity when given subcutaneously. As little as 20 μmoles/DMSA/kg administered sc, according to the stated regimen, protects against the lethal actions of lewisite. Thus, DMSA and DMPS are analogous to BAL not only in chemical structure but also with respect to anti-lewisite activity. In addition, DMSA and DMPS have anti-lewisite activity when given orally (Table 2).
## TABLE I

The Anti-Lewisite Activity of Meso-Dimercaptosuccinic Acid and 2,3-Dimercapto-1-Propanesulfonate when given sc to rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>μmols/kg</th>
<th>survive/start</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LEW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LEW + 75.0 DMSA</td>
<td>12/12</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>LEW + 37.5 DMSA</td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>LEW + 20.0 DMSA</td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>LEW + 10.0 DMSA</td>
<td>1/6</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>LEW + 75.0 DMPS</td>
<td>10/12</td>
<td>83</td>
</tr>
<tr>
<td>7</td>
<td>LEW + 37.5 DMPS</td>
<td>5/6</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>LEW + 75.0 BAL</td>
<td>8/12</td>
<td>67</td>
</tr>
<tr>
<td>9</td>
<td>LEW + 37.5 BAL</td>
<td>3/6</td>
<td>50</td>
</tr>
</tbody>
</table>

---

In the tables of this paper, the data represent the combined results of a number of separate experiments. This was done to save space. The reason for the number of animals in some groups differing from the number in other groups of the same table is that very often the combined data are the result of from 2-3 separate experiments. Otherwise, the experiments were performed under identical conditions. The survival recorded in this table is that for 7 days after lewisite administration.

Lewisite (29.7 μmols/kg) was given sc at time zero.

All agents given sc except BAL, which was given im. Dimercapto compounds given at +1 min, +90 min, +180 min, +360 min after lewisite and at 8 a.m. and 4 p.m. on day 2 and 3. Administration of these amounts of dimercapto compound at the times cited above did not cause any fatalities in control animals that did not receive lewisite (data not shown).

Pair-wise comparisons: p < 0.0001 for 1 vs 2; p = 0.0001 for 1 vs 3 and 1 vs 4; p < 0.001 for 1 vs 6 and 1 vs 7; p = 0.001 for 1 vs 8 and 0.01 for 1 vs 9.
Meso-Dimercaptosuccinic Acid or 2,3-Dimercapto-1-Propanesulfonate is effective, when given by mouth, in protecting rabbits against the lethal effects of Lewisite.

**TABLE II**

<table>
<thead>
<tr>
<th>Group</th>
<th>µmols/kg</th>
<th>survive/start</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 LEW</td>
<td></td>
<td>0/12</td>
<td>0</td>
</tr>
<tr>
<td>2 LEW + 400 DMSA</td>
<td></td>
<td>5/6</td>
<td>83</td>
</tr>
<tr>
<td>3 LEW + 200 DMSA</td>
<td></td>
<td>4/6</td>
<td>67</td>
</tr>
<tr>
<td>4 LEW + 400 DMPS</td>
<td></td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>5 LEW + 200 DMPS</td>
<td></td>
<td>4/6</td>
<td>67</td>
</tr>
<tr>
<td>6 LEW</td>
<td></td>
<td>1/6</td>
<td>17</td>
</tr>
<tr>
<td>7 LEW + DMSA</td>
<td></td>
<td>4/6</td>
<td>67</td>
</tr>
<tr>
<td>8 LEW + DMPS</td>
<td></td>
<td>1/6</td>
<td>17</td>
</tr>
</tbody>
</table>

a Lewisite (29.7 µmols/kg) was given sc at time zero.

b Dimercapto compounds given po at -45, -2, +90 and +300 min. after lewisite and 8 a.m. and 4 p.m. on day 2 and 3. No fatalities occurred in control animals that received these amount of dimercapto compound, po, (but no lewisite) at the times cited above. Survival was followed and recorded for 7 days after lewisite administration.

c Dimercapto compounds given po as follows: 400 µmols of dimercapto compound /kg at 5 min before lewisite, and 200 µmols/kg at each of the following times after lewisite: 1 hr., 2.5 hrs. and 5 hrs. on the first day plus 8 a.m. and 4 p.m. on day 2 and 3.

d For pair-wise comparison: p = 0.001 for 1 vs 2; p = 0.01 for 1 vs 3; p < 0.001 for 1 vs 4 and p = 0.01 for 1 vs 5

Additional studies have demonstrated that a single po administration of DMSA (400 µmole/kg) 15 min prior to lewisite was ineffective since only 1 of 6 animals survived for 7 days. In the experiments of Table 1 and 2, most of the rabbits that received lewisite and no dimercapto therapy died within 12
hrs. If animals died after receiving lewisite plus dimercapto therapy, they usually died between the first and fifth day of the experiment.

**Stability Studies**

The stabilities of DMSA, DMPS and BAL in 0.10M solutions at pH 5.0 and 7.0 were examined (Fig 1). The mercapto groups of these compounds, in aqueous solutions at pH 5.0, are stable (Fig 1). Even after 7 days at room temperature, from 78 to 87% of the mercapto groups remain titratable. At pH 7.0, however, the greater stability of DMSA is evident with 82% of the mercapto groups remaining after 7 days.
Stability of DMSA, DMPS and BAL at pH 5.0 or 7.0. Aqueous solutions of each compound were prepared, adjusted to pH 5.0 or 7.0 and to a final concentration of 0.10mmol/ml. Solutions were prepared using double-distilled H₂O and maintained at 24°. Aliquots were removed at indicated times and the mercapto content determined. Each value shown is the average of two separate titrations.

By this time and under these conditions none of the mercapto groups of DMPS or BAL remained. Other studies (data not shown) indicated that DMSA, in a solution of 5% NaHCO₃, when either frozen for 4 days or frozen and thawed each day for 4 days retained 82% of its original mercapto groups. If similar solutions were held at 4° or 24° for 4 days, DMSA retained 76% and 69% of its mercapto groups, respectively. Although solutions of DMPS in 5% NaHCO₃ were stable (92-95% of original) when either frozen, or frozen and thawed each day.
for 7 days, after three days at room temperature no titratable mercapto
groups were evident.

Discussion

Extensive clinical experience with DMSA and DMPS as antidotes and
prophylactics for mercury, lead, arsenic and other heavy metals has been
reported in Soviet and mainland Chinese literature (10,11,12,13). In the
Soviet Union, DMPS has been for many years an official drug called Unithiol.
Recently, there has been a great deal of interest in both of these water
soluble chemical analogs of dimercaprol in the United States and abroad
(14,15,16,17,18). This has resulted in the confirmation and extension
(19,20,21,22) of earlier reports dealing with both the basic and clinical
investigations of DMSA and DMPS.

Although dimercaprol is a name relatively easily identified in the field
of therapeutics, the compound is known most commonly in other areas as
British Anti-Lewisite. It seemed reasonable to expect that a true analog
agonist might also have Anti-Lewisite Activity.

The present experiments clearly show that either DMSA or DMPS will
protect rabbits against the lethal systemic effects of subcutaneously
administered lewisite (Table 1 and 2). Therefore, DMSA and DMPS can be
considered to be not only analogous in chemical structure but also in
anti-lewisite activity. In addition, DMSA and DMPS are effective when given
by mouth; a route not recommended for BAL administration.

The dose schedule for administering DMSA and DMPS was based on a three
day regimen recommended in the literature for the use of these metal binding
agents. Subsequent studies (Aposhian, unpublished) have demonstrated that as
little as one dose of 40 umols/kg of either drug given in one minute after
lewisite will result in the survival of 4 out of 6 rabbits. In addition when
DMSA therapy is delayed until 90 min after lewisite, 6 of 6 rabbits, survived. The purpose of these studies was to determine whether DMSA or DMPS have anti-lewisite activity. No attempt has been made to quantitate their relative efficacy against lewisite.

Not only are these analogs crystalline and readily water soluble, they are less toxic than BAL. The results of a number of different investigations in rodents have led to the conclusion that the acute toxicity of DMSA is less than that of DMPS which is much less than that of BAL (19,23,24,25).

The stability studies (Fig 1) were initiated for two reasons. Many investigators believe that DMSA and DMPS are unstable because of their dimercapto structure. Since solutions of these compounds were being used throughout the day, for example see Table 1 and 2, it has been considered necessary by a number of investigators (17,20) to prepare solutions immediately before use. The stability of solutions of these dimercapto compounds is somewhat surprising since mercapto compounds are usually thought to be readily oxidized.

In addition to many older reports in the Soviet and Chinese literature (10,12,26) dealing with DMPS and DMSA in human therapy, such use has been strengthened by recent papers containing data from clinical investigations. For example, DMSA has been used recently in the treatment of a 46 yr. old man who ingested 2000 mg of arsenic in a suicide attempt (27). Treatment with 300 mg of DMSA every 6 hrs po for 3 days caused an increase in the urinary excretion of arsenic and eventual recovery. DMSA increased the excretion of lead in the urine of smelter workers and was effective in treating the signs and symptoms of lead poisoning (28). The dimercapto compound was well tolerated and no signs of toxicity were evident. The usefulness of DMPS and other metal binding agents in the treatment of mercury intoxication resulting
from the Iraqi mercury disaster has been documented recently (18). DMPS, as DIMAVAL, is now an approved drug in West Germany for the treatment of mercury poison. These two water soluble analogs of BAL, analogous in activity as well as chemical structure, active when given by mouth and of low toxicity, warrant continued investigation as possible replacements for BAL.

REFERENCES FOR PART I

15. G.C. BATTISTONE, R.A. MILLER, and M. RUBIN, In Clinical Chemistry and
Chemical Toxicology of Metals (S. S. Brown, ed.) 221-224. Elsevier Press
(1980).
23. F. PLANAS-BOHNE, B. GABARD, and E.H. SCHAFFER, Arzneim.-Forsch. 30, 3
1291-1294 (1980).
26. D.M. ZISLIN, I.E. GRONISHENIKOVA, G.N. SAMOKHVALOVA, and A.S. VORONTSCVA,
Gig. Tr. Prof. Zabol 12 17-21 (1968).
27. K. LENTZ, K. HRUBY, W. DRUML, A. EDER, A. GASPNER, G. KLEINBERGER, M.
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SUMMARY

DMPS (2,3-dimercapto-1-propane sulfonate, Na salt) is an important water soluble analog of dimercaprol. All investigations of this antidote for heavy metal intoxication have dealt only with the racemic mixture. In the present report, the optical isomers of DMPS have been separated and the arsenic-antidote activity of the levo-rotatory (-) isomer, the dextro-rotatory (+) isomer and the racemic mixture of DMPS have been investigated in vivo and in vitro. The individual optical isomers and the racemic mixture of DMPS are effective equally, in vitro, in preventing the inhibition by sodium arsenite of the activity of mouse kidney pyruvate dehydrogenase complex (PDH). In addition, when PDH is inhibited, in vitro, by sodium arsenite, any of the three DMPS preparations will reverse the inhibition equally well. The in vitro evidence suggests that two molecules of DMPS are required to prevent the effects of one molecule of sodium arsenite. Neither the LD50s nor the ED50s of each of the three forms of DMPS differ significantly when measured i.p. in mice. In addition, there is no striking difference between the effectiveness of the levo- or dextro-rotatory DMPS when given orally to mice challenged with sodium arsenite. Thus, the use of the individual optical isomers of DMPS does not appear to have any advantage over the racemic mixture as an arsenic antidote under these conditions.
DMPS (2,3-dimercapto-1-propanesulfonate, Na salt) is a water soluble analog of dimercaprol (BAL) (Petrunkin, 1956). Not only is it analogous in chemical structure but it is also analogous in its activity as an antidote for lewisite, 2-chloroethenyl-arsenous dichloride (Aposhian et al., 1982). DMPS is an official drug of the Soviet Union (Klimova, 1958) where it is used clinically and is known as Unithiol. It is used in West Germany as an antidote for mercury intoxication in humans and it is receiving increasing attention in the USA, England and Western Europe. A recent review of its pharmacological and therapeutic properties has appeared (Aposhian, 1983). It is effective p.o., s.c. and i.m. as an antidote against heavy metal intoxication, in particular against arsenic, (Luganski et al., 1957; Tadlock and Aposhian, 1980), mercury, (Kostygov, 1958; Gabard, 1976; Clarkson et al., 1981), lead (Anatovskaya, 1962) and gold (Gabard, 1980). Since the number 2 carbon atom of DMPS is asymmetric, two optical isomers or enantiomers of DMPS exist. Heretofore, published reports dealing with DMPS have dealt only with the pharmacological properties of its racemic mixture or its structural isomer, isoDMPS (Mizyukova and Lokantsev, 1960). The biological properties of the optical isomers of DMPS have not been studied.

The optical isomers of other metal binding or chelating agents often differ in their properties and usefulness (Aposhian, 1961). D-penicillamine, for example, is less toxic than L-penicillamine because it has less anti-vitamin B6 activity. Thus, L-penicillamine is a potent inhibitor of cysteine desulfhydrylase while d-penicillamine is not (Aposhian, 1961). The difference in toxicity is one of the reasons that d-penicillamine, and not its L-isomer or racemic mixture, has been the drug of choice for the treatment of hepatolenticular degeneration, Wilson's Disease.
The present paper reports the separation and some of the antidotal properties of the individual optical isomers of DMPS. Their activity, in vitro, in preventing and reversing the sodium arsenite inhibition of the pyruvate dehydrogenase multienzyme complex (PDH) has been demonstrated. Secondly, the LD50, the ED50 and the therapeutic index of each DMPS form have been determined when given i.p., and found to be essentially the same. Finally, the isomers do not appear to differ in their antidotal activity when given p.o.

METHODS

Animals. CD-1 male albino mice were purchased from Charles River Breeding Labs (Wilmington, MA). They were maintained as described previously (Tadlock and Apohshian, 1980). At the time of their use for these experiments they weighed 26-31 g. The amount of sodium arsenite injected was equal to the approximate LD100. The concentration of the NaAsO₂ solution was such that a 25g mouse received 0.050 ml s.c. The dimercapto compounds were dissolved in 0.9% saline immediately before use and the solutions were adjusted to pH 5.5. The concentration of the dimercapto solutions was such that a 25g mouse received 0.20 ml by the intraperitoneal or 0.20 ml by the oral route. For oral administration, the animals were fasted for 16 hrs and curved 18 gauge oral feeding needles, purchased from Popper & Sons, New Hyde Park, N.Y., were used.

Chemicals. DMPS contains 1 molecule of H₂O of recrystallization. The enantiomers and the racemic mixture (DIMAVAL₅) were titrated with iodine in order to measure mercapto content and purity. All the DMPS preparations were judged to be greater than 99% pure by this criterion.
Thiamine pyrophosphate, NAD\textsuperscript{+}, CoA and sodium pyruvate were purchased from Sigma Chemical Co. (St. Louis, MO). [\textsuperscript{1-14}C]-Sodium pyruvate was purchased from New England Nuclear, Boston, MA.

Separation of optical isomers of DMPS

The reaction of dl-DMPS with the optically active base L(+) arginine leads to the diastereomeric salts of DMPS. They can be separated by fractional crystallization in methanol. The pure optical isomers of DMPS can be isolated by treating each of the diastereoisomeric arginine salts with lead acetate followed by decomposition of the resulting lead salts with hydrogen sulfide. The separation of the isomers of DMPS was also successful using brucine as the optically active base. In this way it was possible to substantiate the physical properties of III and IV.

Pb-2,3-Pb-dimercapto-propane-1-sulfonate (Petrunkin, 1956), 49.6 g, was suspended with stirring in 400 ml methanol. The suspension was saturated with hydrogen sulfide. The lead sulfide was removed by filtration and washed twice with 100 ml methanol. To the combined filtrates was added L(+) -arginine, 9.5g, until pH 4.5 was reached. The addition of hydrogen sulfide and the filtration must be carried out quickly. The filtered solution was then evaporated and the residue recrystallized from 250 ml methanol to give 9.2 g of (+) 2,3-dimercapto-propane sulfonic acid, (+) arginine salt (product I) m.p.: 172 - 173\textdegree C; \textit{[\alpha]}_{20}^{20}: + 7.5 \pm 1.0 \text{ (water, C = 1g/100ml)}). Iodometric titration of mercapto groups indicated that the purified material consisted of equal parts of arginine and (+) DMPS.

The mother liquor, from which I was obtained, was evaporated under reduced pressure. The residue was recrystallized from 50 ml methanol to obtain 8.4g of (-) 2,3-dimercapto-propane sulfonic acid, (-) arginine salt (product II). m.p.: 200-202\textdegree C; \textit{[\alpha]}_{20}^{20}: + 0 \pm 1 \text{ (water, C = 1g/100ml)}.
Iodometric titration of SH-groups showed that the composition of the diastereomeric salt consisted of equal parts of arginine and (-) DMPS.

A solution of (+) DMPS- (+) arginine salt, 9.2g in 200 ml of H₂O, was acidified with acetic acid to pH 3. The solution was stirred at 60°C. An aqueous solution containing 14.2g of lead acetate was heated at 60°C and added to the reaction mixture. The yellow Pb-2,3-Pb-dimercapto-propanesulfonate precipitate was filtered, washed thoroughly with acidified H₂O, H₂O and methanol. The lead salt was suspended in 250 ml methanol and decomposed with hydrogen sulfide. The lead sulfide was filtered, washed twice with methanol and discarded. NaHCO₃ was added to the combined filtrates to give a pH of 4.5. The solution was then evaporated under reduced pressure and the residue was recrystallized from 90% ethanol to give 4.5g of (+) DMPS, sodium salt (III). [α]₂₀° + 2.5 ± 1° (water, C = 1g/100ml). Content: 98% SH - iodometric titration

Using the same procedure that was used to separate III, 3 g of (-) DMPS- (+) arginine salt was prepared and hydrolyzed to give (-) DMPS, sodium salt (IV). Yield: 3.7g; [α]₂₀° - 2.5 ± 1° (water, C = 1g/100ml). Content: 98% SH - iodometric titration

Preparation of mouse kidney extract for PDH complex activity. Eight mouse kidneys (about 4 grams) were sliced and washed with deionized water. The tissue slices were stored in a refrigerator for 2 days (Linn et al., 1972). The tissue slices and 4 ml of 0.25 M sucrose were homogenized at 4°C in a Braun Melsungen homogenizer using 20 strokes at 650 RPM. The homogenate was centrifuged for 20 min in the SS-34 rotor of Sorvall RC2-B centrifuge at 3000xg and 4°C. The supernatant was collected and stored at -70°C until needed.
Assay of Pyruvate Dehydrogenase Complex. The reaction mixture for assaying PDH complex was prepared by adding 0.08 ml of Tris-HCl buffer, (pH 8.1), which contained the cofactors, to a test tube containing $^{14}$C-pyruvate and the supernatant of the mouse kidney extract. The final mixture contained 0.10 M Tris-HCl (pH 8.1), 1 mM MgCl$_2$, 0.5 mM CaCl$_2$, 0.5 mM EDTA, 0.2 $\mu$M thiamine pyrophosphate, 2.5 mM NAD$^+$, 2 mM cysteine-HCl, 0.13 mM coenzyme A, and 2 $\mu$M [1-$^{14}$C]-sodium pyruvate (0.5 mCi/mmole) (Kresze and Steber, 1979) in a total volume of 0.24 ml. After incubation at 30°C for 10 min, 0.24 ml of 20% TCA was added. The $^{14}$CO$_2$ produced was determined to measure PDH complex activity. The $^{14}$CO$_2$ was trapped by the method of Palmatier et al., (1970) and counted in a Packard TriCarb scintillation counter.

In vivo studies - Determinations of the LD50 and ED50 of the various forms of DMPS were performed and analyzed statistically by the same methods as outlined in the biological studies and statistical analysis sections of the paper by Aposhian et al., (1981).

RESULTS

Prevention and Reversal of the Inhibition of PDH The inhibition of the PDH enzyme complex activity by sodium arsenite is demonstrated in Fig. 1. To determine, under these conditions, whether the different forms of DMPS can prevent the inhibitory activity, sodium arsenite (0.25 mM) and various amounts of DMPS were incubated with the PDH complex. $^{14}$CO$_2$ production was determined as a measure of PDH activity. The amount of DMPS needed for 100% prevention of the inhibitory activity of 0.25 mM sodium arsenite was found to be about 0.50 mM (Fig. 2). The amount needed for 100% protection is essentially the same regardless of whether the (-)-isomer, the (+)-isomer or the racemic mixture is used. The concentration of dl-(-)-, or (-)-DMPS needed for 50% prevention, a more quantitative value, was found to be 0.24 mM.
Fig. 1. Inhibition of mouse kidney pyruvic acid dehydrogenase complex activity by sodium arsenite. Sodium arsenite, PDH complex (2.3 mg protein of mouse kidney extract) and other constituents of the PDH complex reaction mixture were incubated as described in Methods.
Fig. 2. dl-DMPS or its enantiomers will prevent the inhibition of the activity of mouse kidney PDH complex by sodium arsenite. Sodium arsenite (0.25 mM), PDH complex (2.3 mg protein of mouse kidney extract), and various amounts of DMPS were incubated with the other constituents of the PDH complex reaction mixture at 30°C for 10 mins. ¹⁴CO₂ was determined as described in Methods. The specific activity of the PDH complex extract was 2.17 nanomols CO₂/min/mg protein.
(Fig. 2). It appears that a DMPS:As ratio of 2:1 is necessary to prevent completely the inhibition of the activity of PDH complex under these conditions.

The relative activity of each form of DMPS in reversing the inhibition by sodium arsenite of the PDH complex activity was determined next. The sodium arsenite and PDH complex were incubated first for 5 mins at 30°C; then different amounts of DMPS were added (Fig. 3) and the incubation continued for 5 mins. The reversal activity of the different forms of DMPS are equal (Fig. 3). To obtain 50% reversal of the inhibitory properties of 0.20 mM NaAsO$_2$, 0.26 to 0.28 mM DMPS is required; 100% reversal required approximately 0.40 mM DMPS. Since the 50% value multiplied by two is more accurate, it appears that a DMPS:As ratio of 2.7 or more realistically between 2.5 and 3.0 is necessary for 100% reversal of the inhibition by sodium arsenite of the activity of the PDH complex. The three forms of DMPS appear to have equal reversal activity.

Protection, in vivo, against the Lethal Action of Sodium Arsenite. The therapeutic index for the different forms of DMPS in mice was determined by dividing the LD50 of the dimercapto compound by its ED50. The latter value is defined as the amount of dimercapto compound (mmol/kg) protecting 50% of the animals against the lethal effects of 0.15 mmol NaAsO$_2$/kg. In this laboratory, this dose of NaAsO$_2$ killed 100% of the animals. The i.p. LD50 for the three DMPS forms are given in Table 1 and the ED50 and therapeutic index values in Table 2.

In the above experiments, DMPS was given i.p. The antidotal activity of the three DMPS forms was determined when given p.o. also (Table 3). No striking difference in the antidotal activity of (+) or (-)- form was found.
Fig. 3. Reversal by dl-DMPS or its enantiomers of the inhibition of the PDH complex by sodium arsenite. PDH complex reaction mixture (0.24 ml) containing 2.58 mg of mouse kidney extract protein and 60 nmol of sodium arsenite were incubated at 30°C for 5 min. Then 0.06 ml of a solution containing 0.1 M Tris-HCl buffer (pH 8.1), 2 mM pyruvate, cofactors and DMPS was added and the incubation continued for 5 more mins. The reaction was stopped with 0.30 ml of 20% TCA. The specific activity of the PDH complex extract was 2.47 nanomols CO₂/min/mg protein.
<table>
<thead>
<tr>
<th></th>
<th>LD50 (mmol/kg)</th>
<th>95% confidence interval</th>
<th>No. of mice used</th>
<th>Dose range (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-DMPS</td>
<td>5.88</td>
<td>4.676-7.214</td>
<td>85</td>
<td>4.0-3.3</td>
</tr>
<tr>
<td>(+)-DMPS</td>
<td>6.02</td>
<td>5.121-6.490</td>
<td>76</td>
<td>4.0-10.0</td>
</tr>
<tr>
<td>dl-DMPS</td>
<td>6.53</td>
<td>5.494-7.706</td>
<td>88</td>
<td>4.0-13.3</td>
</tr>
</tbody>
</table>
TABLE 2. ED50 and therapeutic index of the optical isomers and racemic mixture of DMPS for protecting mice against the lethal action of 0.15 mmol sodium arsenite/kg.

<table>
<thead>
<tr>
<th></th>
<th>(-)-DMPS</th>
<th>(+)-DMPS</th>
<th>dl-DMPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED50 (mmol/kg)a</td>
<td>0.045</td>
<td>0.051</td>
<td>0.055</td>
</tr>
<tr>
<td>Confidence interval</td>
<td>(0.006-0.0824)</td>
<td>(0.0084-0.0877)</td>
<td>(0.0261-0.0820)</td>
</tr>
<tr>
<td>Therapeutic index</td>
<td>131</td>
<td>118</td>
<td>119</td>
</tr>
</tbody>
</table>

a The dose range of DMPS to obtain the ED50 value was 0.012 to 0.120 mmol/kg and the number of animals used was 140 for each DMPS form. DMPS given i.p.; sodium arsenite given s.c.
TABLE 3. DMPS enantiomers are active p.o. in protecting mice against sodium arsenite lethality.

<table>
<thead>
<tr>
<th></th>
<th>7-day survival&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. surv./no. start</td>
</tr>
<tr>
<td>(-)-DMPS</td>
<td>11/16</td>
</tr>
<tr>
<td>(+)-DMPS</td>
<td>9/16</td>
</tr>
<tr>
<td>dl-DMPS</td>
<td>14/16</td>
</tr>
<tr>
<td>(H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>0/16</td>
</tr>
</tbody>
</table>

<sup>a</sup> DMPS (0.12 mmol/kg) or H<sub>2</sub>O was given p.o. 15 min prior to NaAsO<sub>2</sub> (0.15 mmol/kg) s.c. Control animals receiving DMPS (0.12 mmol/kg) p.o. had 100% survival.
DISCUSSION

In the classical work on arsenic, lewisite and dimercaprol by Peters and co-workers (Stocken and Thompson, 1949), pyruvate oxidase was believed to be the most sensitive enzyme. More recent studies have shown that pyruvate oxidase is not a single enzyme. It is known now as the PDH multienzyme complex. The oxidative decarboxylation of pyruvate catalyzed by the pyruvate dehydrogenase complex can be summarized as follows:

\[ \text{Pyruvate} + \text{NAD}^+ + \text{CoASH} \rightarrow \text{CoASAc} + \text{NADH} + \text{H}^+ + \text{CO}_2 \]

The multi-enzyme complex contains many copies of each of the three enzyme components: pyruvate dehydrogenase, (Hamada et al., 1976), dihydrolipoyl transacetylase, (Hamada et al., 1975), and dihydrolipoyl dehydrogenase (Sakurai, et al., 1970). In addition, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase (Baxter et al., 1978; Linn et al., 1972) are present in the complex.

The present experiments using the PDH multienzyme complex activity demonstrates that DMPS can prevent and reverse the sodium arsenite inhibition of this enzyme activity. All three forms of DMPS are equally active in this respect. It suggests that 2 molecules of DMPS are required to prevent the inhibitory activity of 1 molecule of arsenite while somewhat more is necessary to obtain reversal (Figs. 2 and 3). It does not appear that the simple classical picture of chelate or ring formation with the resulting thioarsenite type of structure is applicable, in this case. The ring chelate would be expected to have a 1:1 ratio of chelator to As. We are trying to isolate the DMPS-arsenic structures in order to determine their nature. The three forms of DMPS have similar LD50 and ED50 values in mice. The therapeutic index of each form as a sodium arsenite antidote is independent of stereoisomeric structure (Table 1 and 2).
It has been reported that DMPS is not completely absorbed from the GI tract (Gabard, 1978; Wiedemann et al., 1982). The extent of absorption has been reported to be anywhere between 30-60% depending on the species used. The activity of the DMPS enantiomers in protecting against arsenite lethality was studied p.o. to determine whether the 30-60% absorption of DL-DMPS might be due to one of the isomers in the racemic mixture not being absorbed from the GI tract. If this were so, that isomer when given p.o. would not protect the animal against sodium arsenite. This does not appear to be the case (Table 3). There does not appear to be any striking difference between the individual enantiomers of DMPS as far as their activity p.o. under these conditions.

Very little is known about the biotransformation of DMPS or its isomers. Preliminary studies have indicated a possible conversion to the disulfide and tetrasulfide in biological systems (Luganski and Loboda, 1960). Whether each of the optical isomers of DMPS would be oxidized enzymatically at the same rate is not known and must wait for more sophisticated methods of separation and analysis that are not available at present. These methods are at present being developed in this laboratory. When such methods are found and perfected, additional studies of the optical isomers of this important water soluble metal binding agent that is effective orally should be of interest.
REFERENCES FOR PART II


GABARD, B.: Removal of internally deposited gold by 2,3
dimercapto-propane sodium sulfonate, (Dimaval). Br. J. Pharmacol. 69:

HAMADA, M., OTSUWA, K-I., TANAKA, N., OGASAHARA, K., KOIKE, K.,
HIRAOKA, T., and KOIKE, M.: Purification, Properties, and Subunit
Composition of Pig Heart Lipoate Acetyltransferase. J. Biochem. 73:

HAMADA, M., HIRAOKA, T., KOIKE, K., OGASAHARA, K., KANZAKI, T. and
KOIKE, M.: Properties and subunit structure of pig heart pyrivate


KOSTYGOV, N.M.: The antidotal action of mercaptosuccinic acid and
Unithiol against mercury. Farmakol. Toksikol. (Moscow) 21: 64-69,
1958.

KRESZE, G.B., and STEVER L.: Inactivation and disassembly of the
pyruvate dehydrogenase multienzyme complex from bovine kidney by
limited proteolysis with an enzyme from rat liver. Eur. J. Biochem.

LINN, T.C., PELLEY, J.W., PETTIT, F.H. HUCHO, F., RANDALL, J.C., and
REED, L.J.: Ketone Acid Dehydrogenase Complexes. XV. Purification and
Properties of the Component Enzymes of the Pyruvate Dehydrogenase
Complexes from Bovine Kidney and Heart. Arch. Biochem. Biophys. 148:

LUGANSKII, N.I., LOBODA, Y.I.: Transformation of unithiol in the body.


PART III -
Answers to possible questions as to scope and progress of contract

Questions to be answered about scope and what was and what was not done during the contract from February 1, 1980 to October 31, 1981.

Why didn't we test more compounds? One page 21 of contract proposal dated November 26, 1979, we stated that we would test 23 compounds for protective activity against the lethal activity of sodium arsenite. We actually tested only compounds numbered 1, 2, 5, 7 and 10 of that list. Therefore 5 compounds on the list were tested. We also tested 9 other compounds not on the list. Seven of these are published in Table 3 of our paper that appeared in Toxicology and Applied Pharmacology, 61, 385-392 (1981) and in our first annual report.

Therefore we tested 14 compounds. Why didn't we test the other compounds on the list? For two reasons. First of all, the protective activity of DMPS and DMSA far surpassed our expectation. Because DMPS and DMSA were so successful, it was decided by conversation with Army people that we should push ahead and do LD50, ED50 and therapeutic index studies and compare the effectiveness of DMPS and DMSA. These studies were not even alluded to in the original proposal. They are costly both in number of animals, personnel and computer time and evaluation. Once the effectiveness of DMPS and DMSA were found, these 2 compounds became the primary interest of the PI and his lab. It was deemed very important that their anti-Lewisite activity be determined. The compounds, DMPS and DMSA, were studied by the PI at Edgewood and were found to have anti-Lewisite activity.

Why were the other compounds of Table 1 not tested? There are many reasons: 1. We became more sophisticated and realized that monothiols would not work. 2. We tried polythiols that we did not even know existed at the time of our original proposal.

In summary, looking at page 37 of our original contract proposal, two phases of research were proposed under methods Phase I and Phase II. Under Phase I we assayed 5 of 23 compounds and 9 others that were not listed. We did not study mixed ligand chelates because the effectiveness of mixed ligand chelates in treatment of heavy metal poisoning was retracted by Shubert.

Phase II, Part i -- DMPS and DMSA were shown in preliminary experiments at Edgewood to protect pigs against Lewisite burns. Experiments were tried with mice and Lewisite at Edgewood but they were not successful because of factors out of the control of the PI.

Phase II, Part ii and Part iii were not performed because we ran out of money.

It should be kept in mind that the PI showed that DMPS and DMSA have anti-Lewisite activity. There were no such reports of this activity in the world literature.
The PI is told that $109,000 was originally awarded for this contract and the contract from February 1, 1980 to October 31, 1981 spent a total of $213,000. The difference between what was originally awarded and the total spent was primarily for the purchase of equipment. The purchase of new equipment was justified in letters to the manager of Area V research and/or the COTAR for this contract. These letters as justification for equipment needs are in the Army's files.

It also should be kept in mind that the work from February 1, 1980 to October 31, 1981 in Aposhian's lab was carried out by the PI, Mrs. Boxhorn and 2 honor students. The two honor students worked a minimum of 20 hours a week without salary and were indispensable to the work covered by the original contract.

In addition one member of a site team has criticized us for using mice from Temco Lab and mice from Charles River instead of sticking to one source. We started with Temco mice since the company is located near us in Houston, Texas but we soon found them to be very unreliable as far as ability to deliver. We continued to use them even though it was inconvenient until a logical point came where future experiments would not depend on having the same strain of mice. We then switched to Charles River mice. There has been absolutely no indication that the switch changed any results in either quality or specificity of data.
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