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PROTECTION OF MICE AGAINST THE LETHAL EFFECTS OF SODIUM ARSENITE BY 2,3 DIMERCAPTO-1-PROPANE-SULFONIC ACID AND DIMERCAPTOSUCCINIC ACID

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SUMMARY: 2,3 Dimercapto-1-propane-sulfonic acid, used by the Soviets since 1956 and virtually unknown in the United States, is a water soluble analog of British Antilewisite. 2,3 dimercapto-1-propane-sulfonic acid and dimercaptosuccinic acid are active orally for the protection of mice against the lethal effects of sodium arsenite. They are effective whether given before or after the administration of $NaAsO_2$. Although D-penicillamine and N-acetyl-DL-penicillamine are useful in the treatment of poisoning by other heavy metals, they are devoid of any protective action under these conditions.

INTRODUCTION

There has been substantial recent concern about the medical and biological effects of arsenic as an environmental pollutant (1). As the levels of pollution increase, a need is anticipated for effective therapeutic agents to treat intoxication in humans and farm animals. In addition, severe arsenic poisoning of children by single ingestions of rodenticides, herbicides or insecticides containing arsenic is not uncommon, and often is lethal (2). Since the 1940's, however, BAL* has remained the drug of choice for the treatment of arsenic poisoning (3) even though it has many disadvantages and is far from the ideal drug (3). For example, it is not useful orally and, when injected into humans, a variety of side effects occur that limit the amount that can be administered. For these and other reasons, a program has been started in this laboratory to develop and evaluate agents that bind or chelate arsenic and might have potential therapeutic value. One of these agents, the sodium salt of

*Abbreviations: DMPS, 2,3 dimercapto-1-propane-sulfonic acid, Na salt; DMSA, dimercaptosuccinic acid; BAL, British Antilewisite or 2,3-dimercapto-propanol; D-pen, D-penicillamine; N-Ac-DL-Pen, N-acetyl-DL-penicillamine

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DMPS*, is a water soluble analog of BAL that was developed in the Soviet Union (4) but is little known in the United States**.

MATERIALS AND METHODS

DMPS, DMSA, BAL, and N-acetyl-DL-penicillamine were purchased from Aldrich Chemical Co. DMPS was obtained also as a gift from Heyl & Co., West Berlin. D-penicillamine was a gift of Eli Lilly & Co. MC&B Reagent Grade sodium arsenite was used.

Male albino mice of the TEX: (ICR) strain were purchased from the Timco Breeding Labs, Houston, TX. When used in the experiments, they weighed approximately 25-30 g. Food (Wayne Lab-Blox) and tap water were available ad libitum. However, if the chelating agent was to be given orally, the animals were fasted for the previous 12 hours. The animals were maintained at 22 C with 12 hours of alternating light and dark. The amount of $NaAsO_2$ injected was equal to the approximate LD_{100}. The concentration of the $NaAsO_2$ solution was such that a 25-g mouse received 0.050 ml. The water soluble thiol compounds were dissolved in 0.9% saline immediately before use and the solutions were adjusted to pH 5.5. BAL was dissolved in corn oil. The concentration of the thiol solutions was such that a 25-g mouse received 0.10 ml by the intraperitoneal or oral route. For oral administration, curved 18 gauge oral feeding needles, purchased from Popper & Sons, New Hyde Park, N.Y., were used. The experiments were performed on different days, with different batches of animals, to confirm and extend the results of previous experiments.

RESULTS

None of the mice injected with $NaAsO_2$ and saline survived (Table 1). The deaths occurred within 48 hours after arsenic administration. DMPS and DMSA were found to be potent protective agents against the lethal action of sodium arsenite (Table 1) when either agent is given intraperitoneally immediately after $NaAsO_2$. However, two other well-known, medically useful chelating agents (5), D-penicillamine and N-acetyl-DL-penicillamine, do not protect (Table 1) under these conditions. The results with these two sulfhydryl compounds are unexpected since there have been two reports of the usefulness of penicillamine in the therapy of arsenic poisoning of

**When DMPS references are requested via Medline or Toxline, all but a few of the large number of citations are from Russian and Eastern European journals. Since English translations are not available, we have had to depend on independently acquired translations over which we have no quality control. Since we prefer to cite only references that we have been able to obtain and read, there is an intentional, but not malicious, paucity of Soviet and Eastern European references in this report. DMPS is usually called Unithiol in the Soviet and Eastern European literature. Although the synthesis of DMPS has also been reported by a British group (17) as part of a program to synthesize analogs of BAL, we have been unable to find any publications by them dealing with biological activity.

Table 1. Protection by DMPS and other thiols against the lethal effects of sodium arsenite. The $NaAsO_2$ (0.14 mmoles/kg) was injected subcutaneously in the right rear leg. The chelating agent was administered ip immediately after the $NaAsO_2$. The chelating agents at these doses were not toxic, per se, as shown by the following data. When saline instead of $NaAsO_2$ was given, there was 100% survival of animals receiving the following compounds (mmoles/kg) ip: DMPS (0.80); BAL (0.25); DMSA (0.25); D-Pen (0.80); N-Ac-DL-Pen (0.80). There were at least 12 animals in each group.

Thiol Compound (mmoles/kg) ip	Cumulative 21-day survival No. surviving/No. started Exp I	Exp II	Exp III	Exp IV	Survival %
(Saline)	0/12	0/12	0/12	0/12	0
0.80 DMPS	12/12	8/8	12/12		100
0.40 DMPS	12/12				100
0.25 DMPS	12/12	12/12			100
0.14 DMPS		12/12	9/12		87.5
0.07 DMPS			8/12	11/12	79
0.25 BAL	11/12			11/12	92
0.14 BAL		1/12	1/12		8
0.25 DMSA	12/12			12/12	100
0.14 DMSA		12/12	8/12		83
0.07 DMSA			6/12	10/12	67
0.80 D-Pen			0/12		0
0.25 D-Pen		0/12			0
0.80 N-Ac-DL-Pen			0/12		0
0.25 N-Ac-DL-Pen		0/12			0

humans (2). However, the clinical reports were based on symptomatic relief. Objective criteria were lacking. None of the metal binding agents listed in Table 1 is toxic, individually, at these doses, under the conditions of the present experiments (Table 1).

In addition, we have determined that DMPS or DMSA need not be given immediately after $NaAsO_2$. The administration of either one of the compounds can be delayed at least 2 hours and still be effective (Table 2). Of even greater importance for any therapeutic or prophylactic potential is that DMPS or DMSA is effective even when given orally and prior to the administration of the arsenic compound (Table 3). Under the present experimental conditions, they are effective as oral prophylactics against arsenic intoxication.

Table 2. Experimental therapy with DMPS or DMSA can be delayed after arsenic poisoning. All animals received $NaAsO_2$ (0.14 mmoles/kg) subcutaneously in the right rear leg. DMPS and DMSA were given ip. At the start of the experiment, when $NaAsO_2$ was given, there were 10 animals in each group. However, in three of the experimental groups, one animal died before DMPS or DMSA was administered. Therefore, those groups are listed with 9 instead of 10 started.

Dithiol and time after $NaAsO_2$ it was given	Cumulative 21-day survival No. surviving/No. started		Survival %
	Exp I	Exp II	
(Saline)	0/10	0/10	0
0.25 DMPS			
at 60 min	9/10	7/9	84
at 90 min	9/10	9/9	95
at 120 min	9/10	9/10	85
0.25 DMSA			
at 60 min	7/9	8/10	79
at 90 min	9/10	10/10	95
at 120 min	5/10	6/10	55

DISCUSSION

DMSA decreases the mercury content of tissues of mice and guinea pigs intoxicated with mercury compounds (6,7). Graziano et al. (8) have shown that DMSA, when given intraperitoneally to rats receiving a diet containing As_2O_3, increases the excretion of arsenic. Protection against the

Table 3. Prophylactic and oral activity of DMPS or DMSA. The $NaAsO_2$ (0.14 mmoles/kg) was administered subcutaneously in the right rear leg. DMPS or DMSA was given orally fifteen minutes prior to the $NaAsO_2$. The survival of control animals receiving 1.0 mmoles of DMPS per kg and saline, instead of $NaAsO_2$, was 100%.

Thiol Compound (mmoles/kg) oral	Cumulative 21-day survival No. surviving/No. started			Survival %
	Exp 1	Exp 2	Exp 3	
(saline)	0/8	0/10	0/10	0
1.0 DMPS	8/8	8/10		89
0.75 DMPS		8/10		80
0.50 DMPS		6/10	10/10	80
0.25 DMPS		10/10	7/10	85
0.12 DMPS		0/10		0
1.0 DMSA	8/8			100
0.50 DMSA			10/10	100
0.25 DMSA			8/10	80
0.12 DMSA			4/10	40

lethality of arsenic was not determined. The rat, however, is so different from other mammals in its metabolic handling of arsenic that the National Research Council has recommended that rats not be used for arsenic studies (1).

Information about DMPS is not as easily available since at least 99% of the reports appear in Russian and Eastern European journals**. DMPS synthesis was reported by Soviet investigators (4) in 1956. Although DMPS has been extensively and intensively investigated in the Soviet Union during the last 24 years, it has received only limited and relatively recent interest and recognition in Western Europe (9-12). Moreover, there have been only four published reports from American laboratories dealing with the experimental investigation of DMPS and heavy metals and these have appeared relatively recently (13-15).

In rats, only 30-40% of DMPS given orally is absorbed from the gut (11). The absorption is believed to be by passive diffusion. However, except for the cell membranes of the gut, DMPS is not believed to cross cell membranes. The drug appears to be distributed extracellularly, not intracellularly (11). On the other hand, BAL, because of its lipid solubility does act intracellularly. It would be of interest to determine if the combined use of DMPS and small amounts of BAL would be of advantage therapeutically.

The excretion of DMPS in the rat is rapid. The elimination half-life is 19 minutes (11). Such information about DMSA is lacking. DMPS increases the excretion of mercury in rats challenged with either inorganic or organic mercury (9,10). Its efficacy has been compared with other agents used for treatment of mercury poisoning (9). Also, it protects mice against the lethal effects of cadmium salts (14 and H.V. Aposhian, unpublished data), although high doses must be used.

The present experiments demonstrate the effectiveness of DMPS and DMSA in protecting mice against the lethal action of arsenic. There does not

appear to be a great difference between the effectiveness of these two agents under the present conditions. However, it is clear that D-penicillamine and N-acetyl-DL-penicillamine are without beneficial properties against the lethal effects of arsenic under the conditions used in these experiments. Although to our knowledge**, arsenic chelate stability constants have not been determined for DMPS, such constants, as well as the influence of DMPS in stimulating arsenic excretion, would be valuable in designing and determining the most effective chelating agent for therapy of arsenic poisoning in humans.

Results summarized in Table 3 indicate that the DMPS and DMSA, when given orally and prior to $NaAsO_2$, have prophylactic activity in protecting mice against the inorganic trivalent form of arsenic. Investigation of these two dimercapto compounds as protective agents for workers in mines and smelting plants should be considered. It is not uncommon for such workers, as well as residents of surrounding communities, to display some signs or symptoms of arsenic intoxication (16). The prophylactic oral use of these two dimercapto compounds for protection against the effects of arsenic-containing agents used for riot control, agricultural herbicides, and chemical warfare deserves attention and investigation. To our knowledge, DMPS has not been appraised in experimental human clinical situations in the Western hemisphere. Our studies of DMPS and DMSA imply both prophylactic value and post-exposure therapeutic utility, both of which are clinically useful properties. In addition, the use of these water-soluble analogs of BAL as metal binding agents for *in vitro* biochemical studies deserves investigation.

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