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DMSA AND DMPS—WATER SOLUBLE ANTIDOTES FOR HEAVY METAL POISONING

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INTRODUCTION*

This article reviews the pharmacological properties and the uses of two important antidotes for heavy metal poisoning. Meso-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-l-propanesulfonic acid, Na salt (DMPS) are relatively new antidotes—new, that is, to the western world. Although DMSA was introduced originally by Friedheim et al (1) to increase uptake of antimony during schistosomiasis therapy, Liang et al (77) at Shanghai in 1957 were the first to report its effectiveness as an antidote for heavy metallpoisoning. The synthesis and some of the metal binding properties of DMPS were reported in 1956 by Petrunkin from Kiev (3). Shortly thereafter, DMPS became an official drug in the Soviet Union, where it is known as Unithiol (4).

Between 1956 and 1975, DMSA and DMPS were studied extensively, at both the basic science and clinical levels, in the People's Republic of China, the Soviet Union, and Japan. Some of these investigations have been cited and can be found in an earlier review (5). In the USA and western Europe, however, these two compounds received very little attention until recently. A paper by Friedheim & Corvi (6) in 1975, dealing with DMSA for the treatment of mercury poisoning, and the recent production and availability of DMPS from Heyl & Co., Berlin, stimulated investigators to "rediscover" and study these two metal-binding agents. DMSA and DMPS are water soluble chemical analogs of dimercaprol (British Anti-Lewisite, BAL). In contrast to BAL, they have less toxicity, greater water solubility, and limited lipid solubility, and are effective when given orally.

* Important notes are marked.

If levels of heavy metals such as arsenic, lead, mercury, and cadmium continue to increase in the environment (7), the need will increase also for more effective, therapeutically useful antidotes to treat poisoning by these metals. This need might be met, in the future, by either DMSA, DMPS, or both. They are replacing BAL in the experimental laboratory and in some clinical situations, as discussed below.

All of the papers published on DMSA and DMPS cannot be cited in this review. Space limitations have been imposed on an already lengthy bibliography. Many Chinese, Soviet, and Japanese papers have been translated, reviewed, and included. An emphasis has been placed, though, on important articles published since 1975.

GENERAL AND CHEMICAL PROPERTIES

DMSA and the sodium salt of DMPS are available as white crystalline powders. BAL is an oily liquid. Chemical formulas can be found in an earlier review (5). DMPS has been prepared as the racemic mixture (3), dextro-rotatory form and levo-rotatory form (W. Parr, personal communication). Studies using the D- or L- form, however are rare. In the literature and in this article, the abbreviation DMPS, unless otherwise stated, denotes the racemic mixture of the sodium salt of 2,3-dimercapto-l-propanesulfonic acid.

Since DMSA has two asymmetric carbon atoms, the compound exists as the meso form and the *DL* form. Meso-DMSA is easier to prepare, more readily available, and has been used in most published investigations. There is a striking difference between the chemical properties of these DMSA forms. Meso-DMSA (m.p. 210-211°C) is sparingly soluble. It must be titrated with alkali to approximately pH 5.5 to go into solution. Alternatively, it can be dissolved in *5%* $NaHC0_3$. The *DL* form (m.p. 124-125°) is readily soluble in distilled H_20. In the literature and this article, the abbreviation DMSA, unless otherwise stated, denotes the use of the meso form. This author has learned only recently that most of the Chinese studies have involved the synthesis and biological study of the sodium salt of Na- DMSA, not of DMSA per se. In this review no distinction has been made between the two forms.

Solutions of either DMPS or DMSA are remarkably stable for dimercapto compounds (8), especially at acid pH. Other information about the stability of DMPS has been published but without supporting data (9). For example, it has been claimed that crystalline DMPS retains its antidotal activity and does not decompose when heated for 2 h at 140°C and that aqueous water solutions are stable to prolonged heating. One would suspect, however, that trace amounts of iron and other metals must be absent for stability under such conditions.

Procedures for synthesizing DMPS (3, 10), DMSA (11), ^{35}S-DMSA (12, 13), and [2,3-^{14}C]-DMSA (14) have been reported. DMSA and DMPS have been labeled with ^{99m}Tc for use in renal scanning (15, 16). DMPS is manufactured by Heyl & Co., Berlin, who distribute it as DIMAVAL®. DMSA is available from a variety of biochemical specialty firms in the USA. In the Soviet literature, it is called Succimer.

There have been a number of reports dealing with the stability constants of metal complexes of DMSA or DMPS (17, 18). It has been claimed that the greater the stability constant for a given metal ion complex, the greater the mobilization of that ion when the metal-binding agent is given (19). In the case of mercury complexes, however, there does not appear to be any relationship between survival rates of animals and stability constants (20). DMPS forms complexes with heavy metals that scarcely differ in their stability from metal-BAL complexes except for Cd. The Cd-BAL complex is more stable than the Cd-DMPS complex (21). The stabilities of DMSA complexes, based on their stability constants (22), were found to be in the following order: Cd^{2+}, Pb^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, Ni^{2+}. The Cd complex was the most stable; the Ni complex the least stable. The term chelate has been avoided in this review because by definition a chelate is a ring structure. Since the structure of many of the complexes of DMSA or DMPS has not been rigorously proven, the term metal complex instead of metal chelate is used.

DMSA AND DMPS AS ANTIDOTES FOR HEAVY METAL POISONING

Arsenic

It is rather surprising that since the late 1940s, BAL has remained the drug of choice in the USA for the treatment of As poisoning (23). It has many disadvantages, e.g. high toxicity, low therapeutic index, unpleasant side effects, limited water solubility, instability in solution, and the need to administer by im injection. Side effects, including nausea, vomiting, and headache, have been experienced by 50% of the patients receiving BAL. By 1958, however, publications were beginning to appear in the Soviet literature indicating the superiority of DMPS as an antidote for As poisoning (9). By 1965 the effectiveness of DMSA for this purpose was reported in the Chinese and Soviet literature (25, 26)

The ^{76}As content of 12 organs was sharply reduced when DMPS, 30 mgAg, sc, was given to rabbits (27a). At 24 h after DMPS treatment, ^{76}As elimination in the urine was greater in rate and amount. In rats and rabbits (27b), DMPS prevented the lethal effects of many As compounds, e.g., arsenous oxide, sodium arsenite, calcium arsenite, Paris green, neodiarsenol, sodium arsenate, and osarsol, if given within 1 h after the As com-

pound. Structures have been proposed for the soluble As-DMPS complexes formed by DMPS and different arsenic compounds. Classical thioarsenite ring structures connected in certain cases by additional linear DMPS molecules and having, in some cases, a DMPS: As ratio of 3:2 have been suggested.

In a search for better antidotes of arsenic, a series of mercaptoalkanesulfonates were synthesized (28). In addition to DMPS and iso-DMPS, two other compounds were found to be active as As antidotes. They were 2,3-dimercaptopropoxyethanesulfonate, Na salt and 3(l,3-dimercaptoisopropylmercapto)-propanesulfonate, Na salt. When given to rats 15 min after As_2O_3, iso-DMPS gave greater protection than DMPS. Similar results were found in rabbits. The mercapto groups of iso-DMPS are on the first and third carbon atom. Iso-DMPS, however, is less stable during preservation, slightly more toxic, and more difficult to prepare than DMPS.

DMSA is effective as an arsenic antidote in humans (29), mice (30, 31), and rats (26, 32). It is effective po, ip, sc, and im. Although DMSA increases arsenic excretion in rats (26, 32), the rat 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 (33).

The D and L isomers of DMPS have been studied individually and found to be equally active in preventing and reversing the inhibition by sodium arsenite of the activity of mouse kidney pyruvate dehydrogenase enzyme complex, in vitro, and the lethal effects of sodium arsenite in mice (C. A. Hsu and H. V. Aposhian, to be submitted). Neither is there any significant difference between such in vitro and in vivo activities of the meso- and *DL* forms of DMSA (C. A. Hsu and H. V. Aposhian, to be submitted).

Tadlock & Aposhian (30) have reported that as little as 0.07 mmol of DMPS or DMSA per kg given ip immediately after sodium arsenite protects mice against the lethal effects of sodium arsenite. The dimercapto compounds were also active orally. Dimercapto therapy could be delayed for at least 90 minutes after the administration of arsenite.

In one of the few papers comparing DMPS and DMSA quantitatively in experimental therapy of arsenic intoxication, it was shown that either compound, when given ip, increased the LD50 of sodium arsenite in mice by about 4-fold (31). In addition, the ED50 of DMPS or DMSA ip in mice receiving a LD100 dose of sodium arsenite sc was 0.06 mmol/g. The therapeutic index of DMSA was almost 3 times greater than that of DMPS because the LD50 of DMSA is about 3 times greater than that of DMPS (31). A quantitative comparison has demonstrated that DMPS is 28 times more effective than BAL for arsenic therapy in mice (34).

DMSA was found to be useful in the treatment of a 46-year-old man who ingested 2000 mg of arsenic in a suicide attempt (29). Treatment with 300 mg DMSA every 6 h po for 3 days caused an increase in the urinary excretion of arsenic with eventual recovery. DMPS has also been effective in human arsenic poisoning (N. P. Weger, personal communication).

Not only are DMPS and DMSA analogous in chemical structure to BAL, but they are also analogous in their biological activity when used po, im, or sc to prevent the lethal systemic action of lewisite in rabbits (8).

The treatment of arsine (AsH_3) poisoning is quite different from that of other arsenic compounds. Arsine is a gas and a potent hemolytic agent. The recommended treatment for AsH_3 intoxication in the Soviet Union is mercaptid, which is l,2-propanedithiol-3-(p-tolylthioel). Mercaptid is a clear oily liquid that is readily soluble in organic solvents but insoluble in water. It is readily oxidized, of low toxicity and is given usually im as a 40% solution. It has been suggested that its lipotropic properties promote its penetration into the red cells, where it is needed for arsine oxidation and therapy (35). A mechanism for its action has been proposed (35), involving oxidation to a disulfide after injection. The disulfide then oxidizes arsine. The oxidation products are converted to water-soluble cyclic thioarsenites and excreted. A series of thiol compounds have been tested in rats as antidotes for arsenic trioxide and arsine (36). Those active as antidotes for arsenic trioxide were not antidotes for arsine and vice versa.

DMPS and BAL are contraindicated in acute arsine poisoning since they do not inactivate arsine and can create conditions for increasing arsine toxicity (35). The successful treatment with DMPS and mercaptid of an acute case of arsine poisoning in a human has been described (37) recently. It is clear from the Soviet literature that there is not complete agreement as to the use of DMPS for the treatment of arsine poisoning (35, 37).

Lead

Of all the poisonings by various heavy metals, none seems as insidious as the exposure of children to low levels of Pb found in urban environments of the USA (38). It is recognized that blood Pb concentrations of as little as 20-25 μg/100 ml cause irreversible CNS damage in young children (7). The sources of urban Pb remain ontroversial (38). If ever there appears to be a need for the use of a prophylactic against a heavy metal, the protection of urban children against Pb seems one. The first report (40) of the use of DMSA to treat occupational poisoning by metals was from Peking and Shanghai in 1965. DMSA was found to be as effective as $CaNa_2$ EDTA in the treatment of occupational Pb poisoning and as effective as DMPS in the treatment of occupational Hg poisoning, judged by increases in the urinary excretion of the offending metal.

The successful treatment with DMPS of 60 men with chronic Pb poisoning was reported in 1962 from the Soviet Union (41). They were given 250

mg/day for 20 days. The signs and symptoms of chronic Pb poisoning subsided in the treatment group. When Pb acetate was given intraarterially to rabbits and followed 4 h later with DMSA, the urinary excretion of Pb was 10 times greater than that of the control group (22). In addition, DMSA treatment of rabbits with chronic Pb acetate poisoning resulted in a 7-fold increase in Pb excretion; CaEDTA increased Pb excretion 10-fold in another group.

DMSA given sc or po to rabbits previously challenged with Pb acetate not only increased the excretion of circulating Pb but also removed Pb from tissue and bone (42). Also, the disturbances in porphyrin metabolism usually seen with Pb intoxication were prevented (42).

DMSA, D-penicillamine (D-pen), and EDTA have been compared for their influence on tissue Pb concentrations of mice pretreated with Pb acetate (43). DMSA was the most effective in decreasing tissue Pb. In the brain, a critical organ in Pb intoxication, DMSA reduced the Pb content while D-pen was without effect. Further studies using 30 mg DMSA/kg each day for five days showed that DMSA also increased Pb excretion in rats poisoned with Pb acetate (44). In response to DMSA, about two thirds of the Pb excreted by rats appeared in the urine and about one third in the feces. Using BAL however, the ratio of Pb excretion was reversed and fecal excretion was greater. DMSA did not influence the absorption of Pb from the GI tract.

DMSA was given for six days to five lead-poisoned smelter workers (45). The results of the treatment confirmed the earlier studies by the Chinese in 1965 (40) with DMSA and occupational Pb poisoning. Treatment (45) consisted of approximately 8-13 mg DMSA/kg/day on the first day with increases to 28-42 mg/kg/day on the last day. DMSA, given orally, increased Pb excretion and reduced the Pb concentration of the blood from 97 to 43 µg/dl. No side effects or renal toxicity were detected. It was concluded (45) that DMSA seemed "to be safe and effective for the treatment of Pb poisoning." The use of DMSA and DMPS for prophylaxis against experimental Pb poisoning has been studied and found effective (42, 46a). A recent report (46b) indicates that thiamine (vitamin B1) may have a beneficial effect in the prophylaxis and treatment of Pb poisoning in that it prevented the accumulation of Pb in the tissues of calves given toxic amounts of Pb acetate. Combined therapy using thiamine and/or DMSA and DMPS should be investigated.

Mercury

The problem of treating intoxication by methylmercury (MeHg) is of more recent concern than that of mercuric chloride. DMSA, DMPS, and N-acetyl-DL-penicillamine (NApen) have been shown in a number of studies

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to have some beneficial properties in removing MeHg from the mammalian body. The mercury content of the kidney, liver, and brain of mice or guinea pigs exposed to MeHgBr was decreased by posttreatment with DMSA (6). These experiments were extended (47) to show that smaller amounts of DMSA could be used, greater delay before treatment was possible, and DMSA was effective po. In addition, DMSA was shown to be four times more effective than D-pen for increasing the urinary excretion of mercury. Rats poisoned with MeHg preferred to drink water containing DMSA (2.5 mg/ml) rather than water without it (48).

The activities of DMSA, DMPS, and NApen in mobilizing MeHg in the mouse have been compared by Aaseth & Friedheim (49). The mercapto compounds were incorporated in a diet that was fed to mice from 4 to 12 days after MeHg injection. By the 12th day, DMSA therapy decreased the whole body content of Hg to 19% of that found in the untreated controls. NApen and DMPS were less effective as shown by values that were only 47% and 72%, respectively, of the controls. Of paramount importance is the influence of these metal-binding compounds on the mercury content of the brain, the target organ of MeHg. DMSA accelerated Hg elimination from the brain, but DMPS had no effect. Hg in the blood, kidneys, and liver decreased the most in the DMSA group and least in the DMPS group. The cumulative urinary excretion of Hg was greatest in the DMSA-treated mice and least in the DMPS group.

The efficacy of DMSA > DMPS > NApen = D-pen for removing methylmercuric chloride from erythrocytes, in vitro, (50) was confirmed in vivo by Planas-Bohne (51) with rats receiving ^{203}Hg-methylmercury ip. When the animals were sacrificed, the content of ^{203}Hg-MeHg and $^{203}Hg^{2+}$ in the liver and kidney was measured separately. DMSA was most effective in removing the mercurial from all organs except the kidneys, for which DMPS was better. NApen showed only marginal effectiveness. DMSA removed more of the organic Hg while DMPS removed more of the inorganic Hg. A combination of DMPS and DMSA removed mercury from most organs.

There is disagreement about the relative potencies of DMPS and NApen (49, 51). The differences have been attributed to species differences, routes of administration, and doses of metal-binding agents.

The efficacy of some of the treatments of the victims of the 1971-1972 methylmercury poisoning disaster in Iraq has been published at last (52a). The t1/2 of MeHg in the blood was used as an indication of the efficacy. The mean t1/2 values obtained were as follows: no treatment, 63 days; DMPS, 10 days; thiolated resin, 20 days; D-pen, 26 days; and NApen, 24 days. No adverse effects were seen in any treatment group. A conclusion of the study was that the use of these mercury-mobilizing agents is justified for weeks or months after exposure to MeHg. Such a conclusion is important as it is

not known in what length of time, after MeHg exposure, maximum brain damage occurs.

The most effective agent for removing mercury from the brains of rats given ^{203}Hg-MeHg iv was DMSA, which was better than NApen, which was better than D-pen (52b).

Based on experiments in the dog, the mobilization and removal of MeHg by extracorporeal complexing hemodialysis with DMSA appear very effective and most promising (52c). There is general agreement that BAL should be avoided-in treating prganic mercury poisoning because in mice the complexes it forms appear to accelerate the distribution of mercury from blood into tissues (53), in particular the brain.

When MeHg was given to pregnant rats, and 1 day later DMSA treatment (40 mg/kg/day) was started, there was a 70% decrease in the mercury content of the brains of progeny pups compared to controls whose dams did not receive DMSA (54a). Okonishinikova & Rozenberg have proposed the use of DMSA to prevent occupational poisoning in the workers of mercury industries (54b).

Treatment of inorganic Hg poisoning appears less complicated. Of 15 metal-binding agents given ip to rats, DMPS appeared to be most effective in enhancing urinary excretion and decreasing tissue Hg of rats given ^{203}Hg Cl_2 iv (55). The biliary excretion of $^{203}Hg^{2+}$ in rats was increased by DMPS (56). The influence of DMPS and DMSA on the distribution and excretion of mercuric chloride in the rat has been compared (57). DMPS was more efficient in removing inorganic Hg from the body. If maximum tolerated dose is used as the criterion, however, DMSA > BAL > DMPS for increasing the urinary excretion of $^{203}HgCl_2$ according to the Ding group (25). EKGs of guinea pigs and rats demonstrated that DMSA could prevent the cardiotoxicity caused by iv $HgCl_2$ (25). A thiolated resin, which is not absorbed, has been given orally to trap the mercury in the bile. By stopping the enterohepatic recirculation of Hg, the resin increased the fecal excretion (59).

As the importance of the biliary route for the excretion of mercury is increasingly recognized (60), the introduction of N-(2,3-dimercaptopropyl) phthalamidic acid (DMPA) for experimental therapy in the mouse by Yonaga's group (61) is significant. DMPA (75 mg/kg, sc) enhanced the rate of bile flow and the excretion of mercury into the bile in mice given $HgCl_2$. This has been suggested as the mechanism for the action of DMPA (61). After DMPA treatment, fecal excretion of Hg increased dramatically; tissue and blood concentrations of Hg decreased. DMPA was more potent than equimolar amounts of either BAL or *DL-pen* in Hg mobilization and excretion. Further studies with this new dimercapto compound will be anticipated with great interest.

Cadmium

Cadmium is bound firmly to a cytoplasmic protein, metallothionein (MT). Although the precise metabolic role of this unusual protein is unknown, it appears to control the amount of intracellular diffusible Cd. A brief review of Cd poisoning in humans, the status of chelation therapy, and the need for new antidotes have been presented clearly and succinctly as part of a paper on Cd egress from cells (62).

Ogawa's study (63) of the effect of DMSA on the elimination of $CdCl_2$ strongly points out the dilemma as to which assay to use and what conclusions to draw about the effectiveness of antidotes for heavy metal poisoning. A number of chelating agents including BAL and DMSA were compared (63). The metal-binding agents were given to mice once a day for three days beginning immediately after ip injection of ^{109}Cd. Although DMSA decreased the accumulation of ^{109}Cd in the body, the decrease was small. Essentially similar results point out the relative ineffectiveness of DMPS and other thiols in sustaining Cd excretion or in decreasing tissue concentrations of Cd (64). Yet many of these compounds, including DMSA and DMPS, have been shown to protect mice against the lethal properties of Cd compounds (5,65a). Of all the metal-binding agents tested, however, DTPA and TTHA were the most effective in decreasing the amount of ^{109}Cd in the body (63). DMSA and D-pen increased, the amount of ^{109}Cd in kidneys when compared to nontreated controls. The Cd concentration in the femurs also increased 300% after DMSA treatment. Although BAL is not recommended for treating Cd poisoning as the Cd-BAL complex is believed to be more toxic than Cd alone, the Cd content of the kidneys of the BAL-treated group was decreased to 22% of the control. Recently, Cantilena & Klaassen (65b) have shown DTPA, EDTA, and DMSA to be the most effective of seven metal-binding agents in increasing urinary Cd and reducing tissue Cd when given immediately after Cd to mice. Unfortunately, in clinical situations the need for antidotes is usually at a time long after exposure to Cd, e.g. in "itai-itai" disease in Japan. Cd is considered an essential causative factor in this disease (65c).

The administration of meso-DMSA caused a 9-fold increase, but *DL*-DMSA caused a 26-fold increase in ^{115}Cd excretion (66) when given to rats. Although there was a slight difference in their effects on ^{65}Zn and ^{60}Co excretion, the excretion of ^{64}Cu, ^{59}Fe, and ^{54}Mn was not affected.

Since Cd is bound to cellular MT, an important question has been asked by Cherian (67) as to whether chelating agents will mobilize Cd, which is bound to MT, into the bile. Of a number of metal-binding agents tested, including DMPS, only BAL was effective in this way in rats. As DMPS is a BAL analog, this observation is surprising, but can perhaps be explained

on the basis of lipid solubility. These studies were extended further to investigate the structure-activity relationships that influence the efficiency of metal-binding agents in removing Cd from MT (68).

The synthesis of oligopeptides that contain three cysteine residues and were identical to some sequences of MT has been reported by Yoshida et al (69). The synthetic oligopeptides had a strong affinity for Cd^{2+} and Zn^{2+}. The dissociation constants of the peptide-metal complexes were 2-4 orders of magnitude less than those of cystein-metal or dithioerythritol-metal complexes. Mice receiving 6 mg Cd^{2+} per kg had a survival rate of 27%. Mice receiving Cd^{2+} plus oligopeptide had survival rates of 80-100% depending on the synthetic oligopeptide used. Five oligopeptides were synthesized and tested. Two had a stronger affinity for Cd^{2+} than Zn^{2+}. Another one had a stronger affinity for Zn^{2+} than Cd^{2+}, based on titration data. Unfortunately, polypeptides without structural relationship to MT were not tested as controls and no mention was made of the effect on renal function.

A very promising assay, using human epithelial cells that had previously been made resistant to 0.100 mM Cd, has been used to screen six metal-binding agents for their influence on the egress of Cd from cells. DMPS, mercaptosuccinic acigd and meso-DMSA were found to be the most effective with a minimun of toxicity (62).

Copper, Other Metals and Other Uses

There have been reports in the Soviet literature about the usefulness of DMPS in treating Wilson's disease, but the present author has been unable to locate or obtain specific papers. Papers in the Chinese literature (25, 76) mention work by other Chinese investigators indicating the usefulness of DMSA for treating Wilson's disease. In general, it would appear that D-pen is still the drug of choice for treating this disease, which might explain the reluctance of western clinicians to investigate DMSA and DMPS for such patients.

DMPS ip was found to be the most effective of nine metal-binding agents, including DMSA, in decreasing the lethality of $CuS0_4$ in mice (71). When given to sheep loaded with copper sulfate, DMPS increased urinary Cu excretion only 2-fold while D-pen increased it 10-20-fold (72). The LD50 of copper sulfate in rats, however, was raised about 11-fold by either DMPS or BAL, but only 3-fold by D-pen administration (73). The antidotes were given sc two times after $CuS0_4$ administration. DMPS was found to be more effective than 2-mercaptopropionylglycine in treating copper sulfate toxicity (74).

The antidotal effect of DMSA against a large number of metal salts has been investigated by Ding et al (25). The results demonstrated that DMSA protected mice aginst the lethal effects of silver nitrate, arsenic trioxide,

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cadmium sulfate, cobalt chloride, cupric chloride, mercuric chloride, chloroplatinic acid, nickel chloride, zinc chloride, or zinc nitrate. DMSA did not protect against the acute toxicity of ferric sulfate, aluminum chloride, barium chloride, beryllium sulfate, bismuth chloride, chromium sulfate, potassium bichromate, magnesium chloride, manganese chloride, selenium -oxide, tin chloride, triethyl tin sulfate, strontium nitrate, thallium chloride, or sodium tungstate. A measure of the relative antidote activity of DMSA was indicated by its increasing the LD50 of AS_2O_3 11 times, $AgNO_3$ 9 times, $HgCl_2$ 8 times, $NiCl_2$ 6 times, $CuCl_2$ 4 times, $CoCl_2$ 3 times and $CdSO_4$ 2 times. The studies are important as DMSA was studied as an antidote for a variety of metals in one laboratory. The amount of the antidote used (about 4 mmol/kg) was not small.

Gold compounds given for the treatment of rheumatoid arthritis often elicit toxic side effects involving the hemapoietic system, dermatitis, and nephrosis. Although BAL has been used as an antidote, it is far from satisfactory. Male rats were injected iv with a gold compound and 30 min later were given DMPS (0.75-3.0 mmol/kg) (75). The urinary excretion of gold was increased. The gold content of 5 out of 8 tissues examined was markedly reduced when excised 24 h later.

When mice were given ^{125}Sb, ^{90}Sr, ^{204}Tl, or ^{147}Pm salts followed by DMSA, there was a six-, two-, 11-, or 12-fold increase, respectively, in the urinary excretion of the radioactive metal (76). DMSA has been shown to be an excellent antidote for antimonials. It raised the LD50 of tartar emetic 16-fold in mice (77).

DMPS has been shown to have antidotal activity for cobalt (78), antimony (79), Ag (80), chromium (81), and ^{210}Po (82). On the other hand DMPS appears to retard the urinary excretion of uranium (84).

DMPS, DMSA, and a number of other metal-binding agents have been shown to have antidotal effects in treatment of acute $ZnSO_4$ intoxication in mice (84). The amounts of antidotes used seem excessive.

The clinical value of $^{99}Tc^{m}$-DMSA as a static renal imaging agent has been analyzed in 366 patients and found to be useful (85). Similar experiments with DMPS have been reported (16). The therapeutic and antidotal uses of DMPS in a variety of clinical situations have been reviewed recently (86). Most of these uses, except those as heavy metal antidotes, are rather unconventional and have yet to be confirmed outside the Soviet Union.

PHARMACOKINETICS

An excellent review of Soviet investigations of DMPS as of 1958, including preliminary pharmacokinetic data, is available (9). When ^{35}S-DMPS was given to rabbits sc, the maximum blood concentration occurred 30 min after

injection (9). The blood concentration decreased rapidly; t1/2 was 60 min; and by 24 h the blood was free of ^{35}S. DMPS was rapidly eliminated and did not appear to have any cumulative action. However, all work with radioactive DMPS is based on the premise that the molecule is not biotransformed in any manner. Evidence supporting this is meager (27a).

Wiedemann et al (87) elucidated pharmacokinetic parameters of ^{14}C-(1,3)-DMPS that was given to beagle dogs either iv or po. The t1/2 during the terminal elimination phase was 43 min; the apparent volume of distribution vβ was 160 ml/kg; and the plasma clearance was 2.6 ml/min/kg. When DMPS was administered po, the plasma radioactivity reached a peak in 30-45 min. ^{14}C-DMPS, given iv, was eliminated almost entirely by the kidneys (87). When ^{14}C-DMPS was given po, about 60% of an oral dose was absorbed. This is almost double that found for rats (88). When the binding of ^{14}C-DMPS to plasma protein was measured by equilibrium dialysis, it was found to be about 90% for man and about 70% for dogs (87). There is disagreement as to the extent or degree of DMPS binding to plasma proteins (87, 88).

Extensive data concerning ^{14}C-DMPS distribution and excretion in the rat is available (88). The highest concentration of DMPS was found in the kidney, the lowest in the brain. The concentration in the skin was high. No radioactivity was detected in the expired air. Gabard (88) states that DMPS is extracellular; does not enter the cell; and that the absorption (30-40%) when DMPS is given po to rats is due to passive diffusion through the GI mucosa (88). On the other hand, DMPS may enter the cell via an anion transport mechanism (D. B. Wildenauer, H. Reuther, N. P. Weger, personal communication).

Very little has appeared as to the pharmacokinetics of DMSA. Distribution of ^{35}S-DMSA administered sc and po to rats has been reported (13). Based on the percentage of ^{35}S remaining in the gastric contents, it appears that the DMSA rapidly left the stomach. By 15 min after administration, 57% was found in the gastric tissue, and by 30 min 81%. The peak activity in the serum was reached 15 min after sc and 30 min after oral administration. Most of the radioactivity left the blood by two h, and 95% was eliminated from the body by 24 h.

The oral administration of uniformly labeled ^{14}C-DMSA to monkeys resulted in about 16% of the radioactivity being excreted in urine, about 70% in the feces, and about 1.6% as CO_2 (J. A. Tellotson, personal communication). Recovery averaged about 87%. Furthermore, iv administration resulted in 82% of the ^{14}C being excreted in the urine, 0.3% in the feces, and 0.8% as CO_2 in the expired air. The peak ^{14}C concentration in the plasma of the monkeys po occured at about 90 min. The expired CO_2 was a minor but consistent pathway.

Whole body autoradiographic distribution studies of uniformly labeled ^{14}C-DMSA administered to mice have been reported recently (89). About 10 μCi of DMSA (4 mg/kg), having a specific activity of 13.9 mCi/mmol, were administered iv. The highest levels of radioactivity were found in the blood, lung, kidney, skin, and GI contents at early times. Most of the radioactivity was eliminated by renal and hepatic excretion within 24 h. Radioactivity, however, was present in the bone and GI contents 9 h and 24 h after administration.

TOXICOLOGY AND EFFECT ON TRACE METALS

It should be kept in mind that DMPS and DMSA, in one form or another, have been studied for at least 25 years in the Soviet Union or China. They appear to be remarkably innocuous.

Side effects of DMPS have been summarized (90) in a 1979 report based on a 10-year followup of 168 scleroderma patients who received DMPS as their only therapy. The patients were predominantly women, 9-74 years old. They received 5-10 ml of a 5% DMPS solution im daily, some for as many as 780 days. Although 26 patients exhibited allergic reactions to DMPS, it was suggested that these occurred because the patient had a history of allergies. No anaphylactic shock was seen. Nausea was experienced by 11 patients, weakness by seven, vertigo by four, and itching skin by three. Nephrotoxicity has not been seen with DMPS, whereas it is one of the chief disadvantages of D-pen. The occurrence of diuresis after DMPS administration has been reported (91)

In the Soviet Union, DMPS is usually injected at a level of 5 mg/kg for therapy of humans. When the dosage is increased to 100 mg/kg, its effectiveness is increased, but necrotization and ulcerations often occur at the site of the sc or iv injection (92). This has been observed also by the present author when pigs were given 0.20 mmol DMPS/kg, im or sc (H. V. Aposhian, unpublished). No such necrosis was seen with DMSA. When dogs were given 50 mg DMPS/kg, iv, some of them exhibited muscle tremors, tachycardia, dyspnea, vomiting, and defecation (92, 94).

No changes in behavior, weight, or blood composition by single or repeated administration of DMPS have been noticed at 15 and 80 mgAg in cats, dogs, guinea pigs, rabbits, and mice (9). At higher doses, a brief motor excitement followed by lethargy, vomiting, and cramps was sometimes observed. There was an occasional death. In order of their decreasing sensitivity to the toxic effects of DMPS, animals can be ranked as follows: cats, dogs, guinea pigs, rabbits, and mice. Mice, in other words, are least sensitive. The drug did not influence blood pressure when given iv at levels

of 30-300 mgAg to rabbits. Doses above 500 mg/kg caused hypotension. In dogs, the hypotension became apparent at 200 mg/kg (9).

The organs of dogs given 40 or 60 mg DMPS/kg, iv, three times a day for 2 days and twice during the third day have been examined (93). Plethora of the inner organs, especially the kidneys, were noted. However, by the 15th day these changes disappeared. At the higher dose level, fatty dystrophy of some epithelium was noted. Changes in other organs and tissues were not apparent.

Daily treatment of dogs with DMPS for 6 months did not change various parameters in the serum when compared to control dogs. No pathological changes were found by macroscopic and microscopic examination except for hepatic hemopoiesis in the liver of one animal (94). Animals that received the higher dose of 75 mg/kg, iv, twice daily for 10 weeks showed an increase in spleen and liver iron and a decrease in hematocrit, RBC, and hemoglobin content in the blood. A decrease in serum Zn occurred. Information concerning DMPS and a variety of systems studied in classical toxicology investigations such as serum electrolytes, glucose, uric acid, etc, are available (94, 95).

DMSA and DMPS 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 (31, 34).

Using mice the LD50 of DL-DMPS, D-DMPS and L-DMPS, ip, was found to be about 6.53 mmol/kg, respectively (C. A. Hsu and H. V. Aposhian, to be submitted). For meso-DMSA and *DL*-DmSA the ip LD50 in mice were 13.73 and 10.84 mmol/kg (C. A. Hsu and H. V. Aposhian, to be submitted). The last two values are statistically different. Other LD50 data are available in other animals for DMSA (44, 97) and DMPS (9, 34, 95). The LD50 of BAL ip in mice has been reported as 0.73 mmol/kg (96).

Toxicological studies of DMPS in the rat have shown that the cumulative LD50 resulting from injection of DMPS each day for 10 consecutive days was 30.8 ± 0.83 mmol/kg (95). As to chronic toxicity (95), 600 μmol DMPS/kg given po 5 days per week for 36 or 63 weeks to male and female rats did not result in any difference in the gain of body weight between control and DMPS animals. Organs and tissues after autopsy and histological examination were found to be normal. The effect on tissue trace metals is discussed below. The offspring of DMPS-treated animals did not show any abnormalities (95). Development was normal (95).

DMPS has been evaluated for mutagenicity in the Ames Salmonella-microsome plate test. The results were negative (F. Leuschner, personal communication).

Other pharmacological and toxicological properties of DMSA were reported in 1961 (97). Antimethemoglobin activity was demonstrated. No drug-induced gross or histopathologic changes could be detected, when rats and mice were injected with up to 200 mg DMSA/kg ip 5 days/week for 6 months (44). When the serum chemistries or complete blood counts were determined in rats at these times, they did not differ significantly from the controls. Similar results were found after treating dogs po with about 32 mg/kg for one week followed by 105 mg/kg for 22 weeks. The authors concluded that DMSA appears extremely promising as a relatively nontoxic agent for treatment of metal poisoning (44). Dogs given 500 mg DMSAAg daily (5 days/week) for 6 weeks were without any changes in blood chemistries, EKG, liver function, or renal function tests, but vomiting, decreased food intake, and weight loss were observed (76).

Are the distribution and excretion of trace metals that are essential for growth and maintenance of the organism disturbed when DMSA and DMPS are used experimentally or therapeutically? A fair summary of many results seems to be that when used in therapeutically reasonable amounts, neither DMPS nor DMSA appears to change drastically the amounts of trace elements excreted. The greatest effect appears to be on Cu excretion. Vakhnitsky (98) studied a group of workers who had been treated with 5 ml of a 5% DMPS solution twice a day for 2 days. In their occupations, 37 had been exposed to Hg and 34 to Pb. After the DMPS therapy, the average Cu excretion increased 13-fold and Mn excretion 2-fold. There was a small, statistically insignificant increase in Al excretion.

More recently the effect of DMPS on trace metal excretion has been studied under more controlled conditions using the rat (99). A large dose-dependent increase in urinary excretion of Zn and Cu was found during one 24 h period following a single administration of DMPS (0.025 to 1.0 mmols/kg). The administration of as much as 1.0 mmols DMPS/kg did not change the urinary excretion of Fe or Mn.

Chronic treatment of rats with 600 μmol DMPS/kg each day (5 days/-week) for 36 weeks decreased the kidney levels of Cu to about half that of control animals (95). The Zn concentration of livers, kidneys, skin, and intestine did not change. When DMPS treatment was stopped, the Cu concentration of the kidneys increased daily until 6-7 days after the last DMPS dose, when it reached the copper concentration of the kidneys of the control animals.

Szinicz et al (94) used beagle dogs to study the effect of daily treatment with DMPS for a six-month period. The DMPS effect on the copper content of the serum and organs was found to be dose-dependent. The copper

content decreased in the tissues except in the liver of animals receiving low doses of DMPS (2 mg/kg). No effect on the content of Zn, Fe, Ca, Mn, Mg, or Cd was found. In dogs receiving 2 X 75 mg DMPS/kg iv, there was a large depletion of copper in many organs and an increase in the iron content of the liver and spleen.

The effect of seven metal-binding agents on the urinary excretion of trace metals in mice is a valuable comparative study (100), especially since DMSA was included. At the dosage used, DMSA significantly increased only the excretion of endogenous copper. The excretion of Zn, Ca, Mg, and Fe did not increase significantly after DMSA treatment of five Yugoslavian smelter workers poisoned by exposure to Pb (45). A rise, almost 2-fold, of urinary Cu excretion was noted, but the investigators concluded it was not important clinically.

BIOTRANSFORMATION

Incubation of the ^{35}S-DMPS in blood serum of rabbits at 37.7°C followed by paper chromatography indicated that DMPS appears to be oxidized slowly via an intermediate to DMPS tetrasulfide (70). The intermediate was postulated to be a disulfide. There are, however, a number of structures possible for such a disulfide of DMPS, although this question was not addressed. The tetrasulfide of ^{35}S-DMPS has been found also in the urine of rabbits given ^{35}S-DMPS (70). Much of the DMPS was excreted unchanged during the first hour. Urine collected 5 h after DMPS injection contained the tetrasulfide but no DMPS. There was no evidence for the putative disulfide intermediate in the in vivo experiment.

The fate of 1,3,-^{14}C-DMPS injected iv into male rats has been studied by Gabard and Walser (101a). It was concluded that "at least in rats, DMPS is not involved in important metabolic reactions." Evidence for the tetrasulfide (70) was not discussed (101a) although there were a number of radioactive peaks in the nonacidified urine. Although it is possible that there are species differences, it seems more likely that more experiments are necessary to elucidate more clearly the biotransformation of this important metal binding agent.

Studies of the biotransformation of DMSA have not as yet appeared. Because of its structural resemblance to succinic acid, a number of possibilities exist as to possible intermediates and metabolites. As mentioned in the pharmacokinetics section of this review, very small amounts of $^{14}CO_2$ have been found in the expired air of monkeys given ^{14}C-DMSA.

FUTURE NEEDS

There is a paucity of information about the pathways, if any, for the biotransformation of DMSA and DMPS. To some extent, mercapto groups are usually susceptible to oxidation and carboxyl groups to decarboxylations and reductions. With one exception (70), virtually no such conversions have been reported for these compounds. In addition, with the exception of radioactive techniques, there are no analytical techniques available for specific determination of DMSA or DMPS in biological fluids such as serum or urine. Such techniques would be valuable for determining sites of action, conversions, and distributions of the drugs when radioactive forms of the drug either are not available or, more important, cannot be used.

It seems also that the search for highly specific metal binding agents has diminished. This is unfortunate. As new information becomes available from molecular biology and the new biology, our minds must be open to searching and finding new, highly specific metal-binding agents. An example of such a possibility is the study of the antidotal activity of the synthetic fragments of MT that can now be chemically synthesized (69) and genetically cloned (101b). Another example is the use of bacteria and microbial genetics, e.g. Silver et al (102), for rapid screening and understanding of new metal binding agents and their effectiveness or ineffectiveness. The use of microspheres with a high surface area that contain chelating agents specific for some metals has been proposed. The microspheres have been made but not tested in vivo (103). Such novel approaches will be required as modern technology introduces ever newer technological uses of ever newer metallic compounds and complexes. The increasing use of gallium arsenide in new energy technologies is an example of such a development and the resulting need for protection (104).

Finally, the government and medical community should reexamine its objections to the prophylactic use of metal-mobilizing agents. Their objections are stated, partially, in the discussion that follows the paper cited as (7). Obviously, the putative safety of DMSA and DMPS warrants the consideration of the use of these metal-binding agents prophylactically to protect urban young children from lead, and workers in factories, smelters, and mines from heavy metals to which they are exposed. The question of prophylaxis for such conditions, regardless of past decisions, deserves to be reopened. Such prophylactic uses of these agents in humans and experimentally in animals have been reported by Soviet investigators (105, 106, 107 108).

SUMMARY

DMSA and DMPS are water soluble analogs of British Anti-Lewisite. They are effective when given by mouth, sc, im, and ip as antidotes for intoxication by heavy metals. DMPS has been studied extensively in the Soviet Union since 1954, where it is an official drug called Unithiol. Since 1956 in the People's Republic of China and the Soviet Union, DMSA has been investigated. Western workers "rediscovered" DMSA and DMPS around 1975. These two dimercapto compounds are effective in treating poisoning by compounds of arsenic, lead, organic and inorganic mercury, and other heavy metals. They have been used for this purpose in humans and in experimental animals. They have some effects on Cd intoxication, but other metal-binding agents seem to be more beneficial in experimental situations. DMSA and DMPS are readily excreted via the kidney. The t1/2 for DMPS in dogs is 43 min and for DMSA in rabbits is 60 min. DMSA appears to be less toxic than DMPS, which is much less toxic than BAL. For example, the LD50 (mmol/kg) ip in mice is 13.58 for DMSA, 5.22 for DMPS, and 0.73 for BAL. BAL has many disadvantages of which DMSA and DMPS appear to be free.

When used in therapeutic or reasonable experimental doses, neither DMSA or DMPS appears to have any marked effect on the trace metals in the body except that urinary excretion of Cu and Zn increase. The effects on Cu and Zn are dose-dependent and return to normal if the drugs are stopped. Very little information is available concerning the metabolism and biotransformation of these dimercapto compounds. As an arsenic antidote for mice, the therapeutic index of DMSA is 3 times greater than that for DMPS. DMPS is 28 times more effective than BAL in this respect. It appears that DMSA is more effective than DMPS in removing mercury from the body. DMSA appears to remove more organic mercury, whereas DMPS removes more inorganic mercury. A combination of DMSA and DMPS removes mercury from most organs. Except for differences in their LD50 values, it is premature at the present time to state whether DMSA or DMPS is better as a metal-binding and metal-mobilizing agent. The danger to urban children from chronic exposure to lead should stimulate an examination of the feasibility of the prophylactic use of metal-complexing agents, since DMSA and DMPS appear at this time to be quite innocuous.

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