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TOXICOLOGY INVESTIGATION

Plasma and Urine Dimercaptopropanesulfonate Concentrations after Dermal Application of Transdermal DMPS (TD-DMPS)

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Abstract 2,3-Dimercaptopropane-1-sulfonate (DMPS) is a metal chelator approved in Europe for oral or intravenous use for heavy metal poisoning. Transdermally applied DMPS (TD-DMPS) is used by some alternative practitioners to treat autism, despite the absence of evidence for its efficacy. We found no literature evaluating the pharmacokinetics of the transdermal route of delivery or the ability of TD-DMPS to enhance urinary mercury elimination. We hypothesized that TD-DMPS is not absorbed. Eight adult volunteers underwent application of 1.5–3 drops/kg of TD-DMPS. Subjects provided 12-h urine collections the day before and day of application. Subjects underwent blood draws at 0, 30, 60,90, 120, and 240 min after TD-DMPS application. Plasma and urine were assayed for the presence of DMPS. Urine was assayed for any change in urinary mercury excretion after DMPS. One control subject ingested 250 mg of oral DMPS and underwent the same urine and blood collections and analyses. No subject had detectable urine DMPS or increased urine mercury excretion after TD-DMPS. One subject had detectable levels of DMPS in the 30-min plasma sample, suspected to be contamination. All other samples for that subject and the other seven subjects showed no detectable plasma DMPS. The control subject had detectable urine and plasma DMPS levels and increased urine mercury excretion. These results indicate that TD-DMPS is not absorbed. There was no increase in urine mercury excretion after TD-DMPS. Our results argue that TD-DMPS is an ineffective metal chelator.

Keywords DMPS · Chelation · Mercury · Autism

These data were presented in poster/lecture form at the 2011 North American Congress of Clinical Toxicology. Additionally, an abstract on the methodology of the DMPS assay designed by the FDA-DPS was accepted for presentation at the American Association of Pharmaceutical Scientists Annual Meeting and Exposition in October, 2012.

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Introduction

2,3-Dimercaptopropane-1-sulfonate (DMPS) is a metal chelating agent approved in Europe for oral or intravenous use to treat poisoning with heavy metals such as mercury, lead, and arsenic. DMPS is not currently approved by the US Food and Drug Administration (FDA), although it has been

used in the USA to treat acute arsenic poisoning [1]. It is available to pharmacies in the USA, where it may be compounded into various formulations intended for oral, intravenous, rectal, or topical administration.

Some alternative medicine practitioners provide chelation therapy with DMPS as a "treatment" for autism despite a lack of convincing scientific evidence demonstrating its efficacy and despite its potential for harm [2–7]. One of the formulations of DMPS used by some healthcare providers is a topically applied preparation known as "transdermal"-DMPS (TD-DMPS). We could find no scientific literature evaluating the pharmacokinetics of this route of delivery or of the ability of TD-DMPS to enhance urinary elimination of mercury.

We hypothesized that TD-DMPS is not absorbed into the body, and, therefore, produces neither urine nor plasma levels similar to those reported with oral or IV DMPS dosing, nor does it increase urinary mercury excretion.

The goal of our study was to investigate this hypothesis by assessing whether TD-DMPS produces blood or urine DMPS concentrations similar to those achieved with therapeutic parenteral or oral DMPS dosing, and whether TD-DMPS increases urinary mercury excretion.

Patients and Methods

Patients and Materials

The study was approved by our institutional review board and registered at www.Clinicaltrials.gov. Eight healthy adult volunteers were asked to eat at least three weekly servings of seafood in the weeks prior to the study to increase our ability to detect changes in urine mercury excretion with chelation (a previous study demonstrated that subjects consuming three servings of fish per week had greater increases in urine mercury after oral meso-2,3-dimercaptosuccinic acid (DMSA) chelation than non-fish eaters) [8]. The data collection period lasted 58 days.

The TD-DMPS was obtained from a US compounding pharmacy. The price was $105 per 30-mL bottle of anhydrous gel. The gel was reported to contain 1 mg DMPS and 4 mg glutathione per drop to "enhance transdermal delivery."

The TD-DMPS was analyzed at the FDA Division of Pharmaceutical Analysis and found to contain 0.84 mg DMPS/drop. The product was refrigerated during the study course. The compounding pharmacy reported that it had a shelf life of 90 days, so the TD-DMPS was reanalyzed at the end of the study (71 days after the first subject was treated and 90 days after initial receipt) and found to contain 0.76 mg DMPS/drop. As both values were within experimental error (SD=0.04), it can be concluded that there was minimal change in the product during the study course.

Study Protocol

Each subject provided a 12-h urine collection the day before TD-DMPS application. Urine was collected in a plastic, acid-washed, metal-free container and refrigerated between voids. All urine collections started between 7 a.m. and 9 a.m.

The following morning, an intravenous catheter was placed, and a baseline blood draw was performed.

The baseline blood draw consisted of 8 mL of blood in two lavender-top (dipotassium EDTA [K_2EDTA]) tubes prior to DMPS application. This was considered time zero, and immediately after baseline sampling, 1.5–3 drops/kg of TD-DMPS were applied to the skin on the arms of the subjects. If the arms became saturated, the remainder was applied to the legs.

TD-DMPS doses were based on the "Buttar Autism Treatment Protocol" [2] designed by the developer of TD-DMPS. This protocol used a dose of 3 mg/kg as the initial "challenge" dose, and 1.5 mg/kg as the "treatment" dose to be applied every other day. This protocol assumes a concentration of 1 mg DMPS/drop, as listed on the bottle. A 1-mL syringe was provided with the product. An average drop dispensed from the syringe that accompanied the product was equal to 0.06 mL. Our analysis revealed that at the beginning of the study, the concentration was actually 0.84 mg/drop, and by the end of the study, this had declined to 0.76 mg/drop. However, in calculating our study doses in drops, we adhered to the 1 mg/drop assumption since this is what patients would do. The Buttar protocol uses a maximum of 60 drops for "treatment" dose and 120 drops for "challenge" dose. For subjects 1 and 2, we used the maximum 60 drop "treatment dose". However, we decided to increase this dose since our subjects were larger than children and we wanted to ensure we were not missing detection by under-dosing. We increased to two drops/kg for subject 3 (without the 60–120 drop max), and then used the three drop/kg "challenge dose" (without the 120 drop max) for subjects 5, 6, 7, and 8. Table 1 describes dosing for each subject.

At time zero, all subjects began a second 12-h urine collection. Four milliliter blood samples were drawn into lavender-top (K_2-EDTA) tubes at 30, 60, 90, 120, and 240 min. A single sample at time 30 min from subject #3 was suspected of having been contaminated, but this was recognized too late for repeat sampling. Urine containers were refrigerated between collections. Subject 8 performed a 24-h post-application urine collection instead of a 12-h collection, and had an additional blood draw at 24 h post-application. All subjects washed off any residual DMPS after the post-application 12-h urine collection was complete.

One additional control subject ingested 250 mg of oral sodium DMPS purchased from Sigma Aldrich. This dose

Table 1 Subjects, DMPS dosing, dental amalgams, and urine Hg excretion

Subject no.	Sex/Age(yr)	Dose TD-DMPS	Dental Amalgams	Pre-DMPS UHg (μgHg/gCr)	Post-DMPS UHg (μgHg/gCr)
1	F/34	1.5 drops/kg (60 drops)	0	1.78	1.81
2	M/30	1.5 drops/kg (60 drops)	12	0.48	0.43
3	M/44	2 drops/kg (160 drops)	3	0.95	0.73
4	M/40	3 drops/kg (210 drops)	5	0.83	0.88
5	M/55	3 drops/kg (280 drops)	0	0.52	0.44
6	M/42	3 drops/kg (300 drops)	0	0.35	0.32
7	F/36	3 drops/kg (180 drops)	3	0.43	0.39
8	M/32	3 drops/kg (190 drops)	0	0.34	0.12
Control	F/42	oral DMPS 250 mg	0	0.90	5.52

TD-DMPS transdermal DMPS, *UHg* 12-h urinary Hg excretion in μg Hg/g creatinine

The control subject ingested 250 mg sodium DMPS per kg

was based on the "challenge" dose of DMPS in a previous study [9]. This subject underwent the same blood and urine collection protocols but did not have the TD-DMPS applied.

Sample Processing

All blood samples were agitated, placed on ice, and processed within 15 min. Processing consisted of centrifugation for 3 min, and then transfer of plasma into plastic, aluminum foil wrapped tubes containing 10 mg of monobromobimane (mBBr) preservative. The headspace was purged with nitrogen and tubes were vortexed for 20 s. Tubes sat at room temperature for 20 min and were then stored at −20 °C.

After completion of urine collection, total urine volume was measured, and three aliquots were removed. Two aliquots of 20–30 mL were used for measurement of urine creatinine and DMPS. A third 3-mL urine aliquot was transferred to a 5-mL Nalgene cryovial containing 30 μL of preservative and used for measurement of urine mercury concentrations. Urine samples were stored at −20 °C until shipping or analysis. Urine DMPS, metabolite, and mercury concentrations were measured at room temperature.

Batched plasma and urine samples were sent overnight on dry ice and remained frozen upon arrival, where they were immediately processed or stored at −80 °C until analyzed. Plasma samples were shipped within 1–14 days of collection. Urine samples were kept frozen throughout the study and sent as a batch at the end. The longest any sample remained frozen before shipping was 84 days.

Sample Analysis

DMPS

Plasma and urine samples were assayed for total DMPS (reduced and oxidized) by the FDA Division of Pharmaceutical Analysis. The method utilized for assessment of the TD-DMPS gel and plasma/urine samples was a modification of the work done by Maiorino and coworkers [10, 11]. All standards were obtained from Acros Organics (Fisher Scientific).

Narrow bore HPLC coupled with fluorescence detection was used for all analyses. DMSA was used as an internal standard for all analyses. mBBr was used as the derivatizing agent for all samples and standards, and dithiothreitol (DTT) was a reducing agent used to break disulfide linkages to generate reduced DMPS. Sample preparation for all types of samples analyzed is provided below.

DMPS/Glutathione Product

To a 13 mm × 100-mm glass tube, 1,800 μL of 0.1 M ammonium bicarbonate, 100 μL of DMPS standard or sample, 50 μL of DMSA solution, and 50 μL of 20 mM mBBr were added. The headspace was purged with nitrogen, capped, and shaken for 5 min. Methylene chloride (2 mL) was added to remove excess mBBr. The solution was centrifuged for 2 min. The organic layer was removed, and the aqueous layer was acidified by adding 17 μL of 6 N HCl to prevent column degradation.

Plasma Samples

Standards were prepared using plasma from a given subject prior to administration of TD-DMPS. To a 13 mm × 100-mm glass tube, 200 μL of plasma standard or sample, 50 μL of DMSA solution, 1,700 μL of 0.1 M ammonium bicarbonate, and 50 μL of 100 mM DTT were added. The solution was mixed vigorously for 1 min and incubated under nitrogen for 30 min. The solution was transferred to an Amicon Ultra microconcentrator and centrifuged at 5,200 gravities (G) for 45 min at 23 °C. The filtrate was transferred to a glass tube, and 200 μL of 80 mM mBBr was added for derivatization. After purging with N_2 for 10 s and vigorous mixing, the contents were incubated for 10 min in the dark at room temperature. The filtrate was extracted twice using 4 mL methylene chloride. After centrifugation at 2,600 G

for 2 min, the organic layer was removed and the aqueous layer was acidified by adding 17 μL of 6 N HCl.

Urine Samples

Standards were prepared using urine from a given subject prior to administration of TD-DMPS. To a 13 mm × 100-mm glass tube, 800 μL of 0.1 M ammonium bicarbonate, 100 μL of urine sample or standard, 50 μL of DMSA solution, and 50 μL of 100 mM DTT were added. The solution was mixed, purged with nitrogen, and reacted at room temperature for 50 min. One hundred microliters of 80 mM mBBr was added and purged with nitrogen and reacted for 10 min. The filtrate was extracted twice using 2 mL methylene chloride. After centrifugation at 2,600 G for 2 min, the organic layer was removed and the aqueous layer was acidified by adding 17 μL of 6 N HCl.

Validation of DMPS Assay

Validation parameters were assessed prior to testing samples from each subject using pre-DMPS plasma or urine. The linearity range assessed was 4–50 μM. Specificity was determined by comparing a blank sample of pre-DMPS plasma or urine and showing no interference in the region of the chromatogram where DMPS would elute. A recovery sample was prepared using the pre-DMPS plasma or urine for each subject to ensure that DMPS could be recovered from each subject's plasma or urine. The recovery amounts for these spiked samples ranged from 89.8 to 112.3 % in plasma and 88.7–117.8 % in urine. The limit of detection and limit of quantitation was determined for each subject per matrix analyzed using the response of the three lowest concentration linearity standards and the line estimation function in Excel (Microsoft, Redmond, Washington). The LOD range in plasma was 0.21–3.61 μM and in urine was 0.98–5.32 μM, depending on the subject. The LOQ range in plasma was 0.62–10.94 μM and in urine was 2.96–16.11 μM.

In addition to the validation measures performed by the FDA, investigators performed one blood draw from a subject who had no exposure to either oral or transdermal DMPS. After the blood was drawn, it was spiked with 500 μL of 0.04 mg/mL DMPS and processed in the same manner as the other specimens. The full DMPS amount was recovered and detected with the assay.

Other Sample Analyses

Urine Mercury

Urine mercury analyses were performed by the Inorganic and Radiation Analytical Toxicology Branch of the National Center for Environmental Health at the Center for Disease Control in Atlanta, Georgia via the analytical method DLS 3002.1, "Urine mercury and iodine by inductively couple dynamic reaction cell plasma mass spectrometry (ICP-DRC-MS)", and had a limit of detection of 0.08 μg/L [12]. The normal reference range for urine mercury is 0.44–2.66 μg/L. The method parameters have been described previously [13]

Urine Creatinine

Urine creatinine concentrations were measured via the enzymatic method with creatininase. All urine mercury results were corrected for urine creatinine to yield results as micrograms Hg per gram creatinine.

Statistical Analysis

Descriptive statistics were used for plasma and urine DMPS concentrations. Pre- and postexposure values of 12-h urine Hg excretion, expressed as micrograms Hg per gram creatinine, were compared using Wilcoxin paired ranks test, with a two-tailed $p<0.05$ considered statistically significant.

Results

Of the 41 plasma samples obtained from eight subjects given TD-DMPS (collected between 30 min and 24 h after DMPS application), DMPS was detected in one sample (Table 2). A level of 2.8 μM was detected in subject 3 at 30 min after TD-DMPS application. This was the sample suspected of contamination during the blood draw. None of the other plasma or urine samples from that subject had detectable DMPS. None of the plasma or urine samples from the other seven subjects contained detectable DMPS at any time point.

The control subject given oral DMPS had plasma DMPS levels measured between 14.3 and 20.5 μM at every time point. Additionally, this control subject had detectable urine DMPS of 40.2 μM.

TD-DMPS application did not result in a statistically significant increase in urine mercury excretion ($p=0.106$, Table 1). The control subject, who ingested oral DMPS, showed a sixfold increase in 12-h urine mercury excretion. None of the subjects reported adverse reactions to TD-DMPS.

Discussion

DMPS is a chelating agent that binds to metals, forming a complex which is renally excreted. It was designed in 1958 in the former Soviet Union as an orally available antidote to

Table 2 Plasma DMPS concentrations (uM) after TD-DMPS application in eight subjects

Subject no.	Dose TD-DMPS	0 min	30 min	60 min	90 min	120 min	240 min	24 h
1	1.5drops/kg (60drops)	0	0	0	0	0	0	N/A
2	1.5drops/kg (60drops)	0	0	0	0	0	0	N/A
3	2drops/kg (160drops)	0	2.8[a]	0	0	0	0	N/A
4	3drops/kg (210drops)	0	0	0	0	0	0	N/A
5	3drops/kg (280drops)	0	0	0	0	0	0	0
6	3drops/kg (300drops)	0	0	0	0	0	0	N/A
7	3drops/kg (180drops)	0	0	0	0	0	0	N/A
8	3drops/kg (190drops)	0	0	0	0	0	0	N/A
CONTROL	Oral DMPS 250 mg	0	15.0	18.5	20.5	18.8	14.3	N/A

A control subject ingested 250 mg DMPS as the sodium salt

[a] This specimen was believed to be contaminated with DMPS during collection (see text)

"Lewisite," an arsenic-containing biological warfare agent. DMPS became available to the Western world in 1978 when the German pharmaceutical company, Heyl, began manufacturing and distributing it as a treatment for arsenic, mercury, and lead poisoning [14]. DMPS is approved in Europe for treatment of heavy metal toxicity, but is not approved by the US FDA for use in the USA.

Chelating agents such as DMPS have received significant attention in recent decades as controversy has arisen regarding a proposed link between mercury and autism [15]. However, after years of study, no causal relationship between mercury present in vaccines and autism has been established [16, 17]. Some practitioners continue to assert that mercury and autism are causally linked and treat patients with chelating agents in an effort to both eliminate mercury from the body and treat the autism [2, 5, 6]. One of the agents used in some autism chelation protocols is a transdermal formulation of DMPS (TD-DMPS).

Pharmacokinetic information is available for the oral and intravenous routes of DMPS administration [18, 19]. A canine study showed peak plasma concentration occurred 30–45 min after oral administration, and plasma half-life was 43 min (after oral or IV administration) during the terminal elimination phase. After parenteral administration, DMPS was almost exclusively eliminated via the kidneys [20]. A human study showed that in subjects given 300 mg of oral DMPS, DMPS was detectable in blood samples in its unaltered or reduced form from 30 min to 4 h, and from 30 min to 24 h in its oxidized, disulfide form. The maximum plasma concentration in this study was (mean) 25.3 μM (SE± 3.0 μM) [18]. In the same human study, both oxidized and reduced DMPS were detected in the urine of subjects for a 24-h period, which encompassed six separate collections. Urinary total DMPS levels peaked around 9.5 h[18]. In contrast, after TD-DMPS, we were unable to detect any reduced or oxidized DMPS in urine.

Scientific studies demonstrating absorption of TD-DMPS through the skin do not exist. The goal of our study was to determine whether topically applied TD-DMPS is absorbed into the body and leads to increased urine mercury excretion. We chose the timing of our assays based on the available data for parenteral and oral DMPS. Though transdermal preparations may be absorbed more slowly than oral ones, the fact that our blood collections at 30, 60, 90, 120, and 240 min, and our 12- and 24-h urine collections all demonstrated no detectable DMPS (except for a single, probably contaminated plasma specimen) provides evidence against any significant skin absorption.

Our assay was validated via several means. This includes the calibration curves created for each individual subject, the blood sample spiked with DMPS after drawing, but before processing, and our control subject who ingested oral DMPS. These mechanisms attest to the robustness of the assay and the strength of our results.

As noted in Table 2, subject 3 had minutely detectable amounts of DMPS in the plasma at 30 min. This subject did not have detectable plasma levels at any other time, nor was any DMPS detected in his urine. Given the robustness of the rest of the data, the detectable DMPS in plasma was likely due to contamination, as was suspected prior to analysis. The gel was oily and viscous, and the investigator drawing the blood was suspected to have inadvertently contaminated the glove and collection tube while drawing the sample.

A potential weakness of this study is that we used the same weight-based dosing for our adult subjects as is used in children. Children have a larger body surface area (BSA) to weight ratio than adults, and could theoretically have higher blood levels due to greater absorptive surface area. Average BSA for a 2-year-old child is 0.5 m^2 (average weight 15 kg), for a 10-year-old child is 1.14 m^2, and for an adult is 1.73 m^2 (average weight 70 kg). Even if children achieved DMPS plasma levels twice our lower limit of detection, it would still be far below the concentrations reported after oral or IV administration of therapeutic doses of DMPS. We do not think weight-based dosing ultimately affected our study results. There is also potential for variation of drop size depending on the particular dispenser used to measure a dose. It seems unlikely such small variations

would affect our results. Although seafood intake was not standardized among subjects, the purpose of encouraging seafood intake in subjects was to increase the likelihood of being able to measure mercury in the urine of subjects and, therefore, detect an effect of DMPS should one exist. Each subject had measureable, but low, urine mercury excretion before and after DMPS, and no subject exhibited an increase in excretion. We do not feel the lack of standardization of seafood intake affected our results.

Many characteristics determine whether a drug is absorbed through the skin. Factors which promote dermal absorption include nonionization at physiologic pH, high lipophilicity, and low molecular weight [21]. DMPS is ionized at physiologic pH. It is a relatively small molecule (molecular weight 228.27 Da) but is very polar. Drug absorption through the skin is also affected by volatility of the compound, temperature, concentration, skin site, and skin integrity [21]. Though we followed the instructions provided by the compounding pharmacy for storage and application of the product, the DMPS concentration in the product did decline slightly during the study period. This decline would also occur during the time period a patient would store the bottle during the usage period. It is instructed that the product be applied to intact skin (which is less permeable than compromised skin).

In addition to assaying for DMPS in blood and urine, we measured urine mercury excretion before and after TD-DMPS administration. We collected pre- and post-DMPS urine specimens starting and ending at the same time of day to control for diurnal variation. If TD-DMPS is absorbed and capable of increasing urinary mercury excretion, we would expect to see some rise in the excretion of urine mercury by the subjects, as we do with oral and parenteral administration of DMPS to healthy volunteers [18, 19]. This did not occur in any of our TD-DMPS subjects. Urine mercury concentration did increase in our control subject, commensurate with rises described in the literature following a "DMPS challenge test" [22]. There have been reports that subjects with mercury containing dental amalgams show a greater increase in urine mercury excretion after DMPS administration [22]. Our control subject had no dental amalgams (see Table 1 for dental amalgams in all subjects).

Conclusion

In our study of eight volunteers who applied commonly recommended doses of TD-DMPS, DMPS was not detected in the blood or urine, indicating that it is not absorbed transdermally. Subjects did not exhibit an increase in urine mercury excretion after dermal exposure to this agent. Our results provide evidence against the use of TD-DMPS as an effective systemic metal chelator.

Conflict of Interest The authors have no financial relationships or conflicts of interest relevant to this article to disclose.

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