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Antidotal Action of Some Gold(I) Complexes toward Phosphine Toxicity

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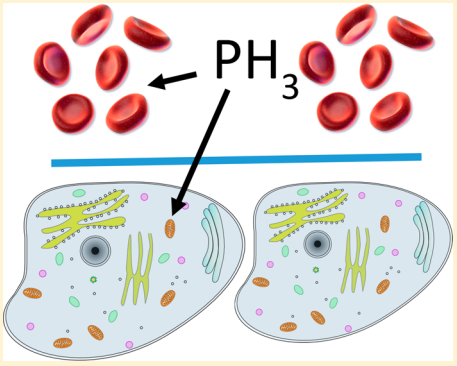
Supporting Information

ABSTRACT: Phosphine (PH_3) poisoning continues to be a serious problem worldwide, for which there is no antidote currently available. An invertebrate model for examining potential toxicants and their putative antidotes has been used to determine if a strategy of using Au(I) complexes as phosphine-scavenging compounds may be antidotally beneficial. When *Galleria mellonella* larvae (or wax worms) were subjected to phosphine exposures of 4300 (±700) ppm·min over a 20 min time span, they became immobile (paralyzed) for ∼35 min. The administration of Au(I) complexes auro-sodium bisthiosulfate (AuTS), aurothioglucose (AuTG), and sodium aurothiomalate (AuTM) 5 min prior to phosphine exposure resulted in a drastic reduction in the recovery time (0−4 min). When the putative antidotes were given 10 min after the phosphine exposure, all the antidotes were therapeutic, resulting in mean recovery times of 14, 17, and 19 min for AuTS, AuTG, and AuTM, respectively. Since AuTS proved to be the best therapeutic agent in the *G. mellonella* model, it was subsequently tested in mice using a behavioral assessment (pole-climbing test). Mice given AuTS (50 mg/kg) 5 min prior to a 3200 (±500) ppm·min phosphine exposure exhibited behavior comparable to mice not exposed to phosphine. However, when mice were given a therapeutic dose of AuTS (50 mg/kg) 1 min after a similar phosphine exposure, only a very modest improvement in performance was observed.



INTRODUCTION

Worldwide, ingestion of pesticides is seemingly the most common method of suicide, accounting for approximately one-third of all such deaths.[1,2] Since the early 1980s, particularly in parts of Asia, phosphine (PH_3) released from pelleted phosphides has become increasingly used as the poison within this genre,[3−8] yet there appears to be no antidote currently available. Throughout North America, phosphides (particularly of aluminum and zinc) are legally obtainable from many commercial outlets in pelleted form for use as rodenticides. There are dozens of sublethal occupational exposures annually in the U.S.[9,10] and occasional domestic accidents leading to fatalities in Canada and the U.S.,[11,12] but of greater public health concern is the possibility that phosphine may be deliberately put to malicious purposes, since the phosphide pellets release the toxic gas simply upon contact with mildly acidic water. A key target for the acute toxic action of phosphine is believed to be the mitochondrion, seemingly by inhibition of cytochrome *c* oxidase (complex IV).[13−17] Unfortunately, rigorous verification of this mechanism of action at the biochemical and cellular levels is lacking, representing a barrier to the rational development of possible antidotes. Phosphine, however, is slow acting, relatively stable in vivo, and a ligand much used in synthetic chemistry; it follows, therefore, that a scavenging approach employing metal ion complexes designed to bind phosphine ought to significantly ameliorate its toxicity.

In this investigation, we studied a number of compounds that are commercially available and have previously been evaluated for their pharmacological activity and safety, although not as decorporating agents. The essential desired activity we sought was the ability to rapidly bind phosphine with reasonably high affinity, and based on general inorganic principles,[18,19] we proposed that some gold(I) complexes should prove to be good candidate phosphine antidotes. Phosphine is a "soft" ligand with a marked preference for binding in σ-donor/π-acceptor fashion to "soft" metal ions, typically second- and third-row transition metals in low oxidation states. Gold in its univalent state, Au(I), is the softest metal ion, and given that Au(I) compounds have been widely used to treat rheumatoid arthritis for about a century,[20,21] we gave Au(I) complexes a high priority for investigation as potential phosphine-scavenging agents. Non-life-threatening side effects develop in about one-third of patients given repeated high doses of Au(I) antiarthritics, but these are usually minor and manageable/reversible.[22,23] Gold salts are about an order of magnitude more expensive than salts

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of most first-row transition metals, but this cost is still small in in comparison to that of the overall purified product.

In addition, we have previously shown that *Galleria mellonella* larvae (caterpillars) can usefully be applied to the screening of antidotes for mitochondrial toxicants, namely, azide, cyanide, and sulfide.[24] Accordingly, we have employed *G. mellonella* larvae to find an exposure level to phosphine gas useful for testing both the prophylactic and therapeutic effects toward phosphine of three antiarthytic Au(I) compounds, namely, auro-bisthiosulfate (AuTS), sodium aurothiomalate (AuTM), and aurothioglucose (AuTG). All of these compounds contain sulfide donors, keeping the gold in its reduced univalent state, lowering toxicity, and promoting affinity for phosphine. The outcomes of these experiments with the larvae were then used to guide the development of a protocol for testing the potential antidotes to phosphine in mice.

EXPERIMENTAL SECTION

Reagents. All chemicals, ACS grade or otherwise stated, were purchased from either Sigma-Aldrich or Fisher and used without additional purification. Aluminum phosphide (AlP) and argon gas purchased from American Elements and Matheson, respectively, were also used as supplied. Gold compound solutions were prepared using phosphate-buffered saline (pH = 7.4). Phosphine gas was generated through the reaction of either calcium phosphide (Ca_3P_2) or aluminum phosphide with sulfuric acid. In the exposure of mice or *G. mellonella* larvae, 20 mL of 1.2 M sulfuric acid was added to ∼0.5 g of Ca_3P_2 to slowly release phosphine in a closed container. The time-dependent variation in phosphine production was observed by infrared spectroscopy at 2325 cm^{-1} (Thermo-Nicholet 6700 FT-IR) using Beer's law to quantitate the measurements. Calibration was by a standard additions method using pure phosphine gas generated by the action of sulfuric acid on AlP, a more rapid reaction than that employed in the inhalation chamber. The exposures to phosphine gas are reported as integrated concentration (ppm) × time (min) of phosphine exposure.

Animal Studies—*G. mellonella* Larvae. Larvae of the Greater Wax Moth, *G. mellonella,* were purchased from Vanderhorst Wholesale, Inc. (Saint Mary's, Ohio) and were acclimated at 25 °C for 6 days. Larvae were randomly selected for groups of 10 organisms each. Groups were exposed to phosphine gas generated from calcium phosphide pellets using sulfuric acid in a sealed container (4.8 L volume) as above. In addition, a group of larvae were exposed to a vessel containg sulfuric acid placed in the inhalation chamber as an added control. Phosphine exposure lasted for 20 min, and the atmosphere was pumped with a 30 mL syringe every 5 min in order to circulate the phosphine. After exposure, the wax worms were removed from the chamber and monitored for recovery from paralysis ("knockdown"). Recovery was determined by the repeated performance of righting behavior as described by Frawley et al.[24] Gold compounds and control solutions were administered at determined time points either before or after phosphine exposure. All injections were a maximum volume of 10 μL and into the most distal left abdominal proleg. Solutions were prepared with filtered PBS, which also served as the injection control. Organisms were monitored for immune activity signaled by a melanin-mediated color change.[25] *G. mellonella* mitochondrial particles (mainly broken mitochondria) were prepared by cooling about 20 larvae to 4 °C for 12 min. Larvae were then minced in 1 mL of EDTA/KCL solution (154 mM KCl, 1 mM EDTA; pH adjusted to 6.8).[26] The minced tissue was then gently homogenized using a glass homogenizer in 5 mL of EDTA/KCl solution, filtered through cheesecloth, and subsequently centrifuged at 1500*g*, 4 °C, for 8 min to collect mitochondrial particles. The pellet obtained was then washed with 200 μL of EDTA/KCl solution, suspended in 200 mL of the same solution, and placed on ice subsequent to use in respirometric experiments.

Animal Studies—Mouse Model. The University of Pittsburgh Institutional Animal Care and Use Committee (Protocol Number 17091400) approved all animal protocols used in this study. The Division of Laboratory Animal Research of the University of Pittsburgh provided all veterinary care during these experiments. Male Swiss-Webster (CFW) mice weighing 35−40 g (6−7 weeks old) were purchased from Taconic, Hudson, NY, housed four per cage, and allowed access to food and water ad libitum. Animals were allowed to adapt to their new environment for 1 week prior to carrying out experiments. All animals were randomly assigned to experimental groups of predetermined size. Animals, two at a time (one subject given test antidote, plus one control), were exposed to phosphine gas in a procedure that was otherwise identical to that used for the *G. mellonella* larvae (see above) for 15 min.

Following phosphine exposure, the duration of time required for the recovery of pole climbing in mice was measured following a procedure originated by Frawley et al.[24] This test evaluates the ability of the mouse to climb a lightly roughened, 24 inch pole (3/8″ diameter) before and after exposure to a toxicant as well as the recovery, post exposure when receiving a treatment. This test is based on the observed natural curiosity of the mouse to climb to the top of the pole; it is relatively simple and requires minimal equipment. The pole test was started at 20 min post toxicant exposure, or as soon the mouse righted itself, and continues every 10 min until the mouse was fully recovered. Full recovery was assessed by the mouse scoring the highest rating possible (3), three times in a row. Briefly, the pole was placed in the horizontal position (45° angle), and the mouse was placed onto the end. The pole was then gradually raised to the vertical position (through a 90° angle). Once the mouse climbed to the top, or not, it was removed from the pole, scored, and replaced in its bucket until the next trial. The mice were scored by performance, receiving scores (see Table 1, Supporting Information) from 0 (fall off/cannot grasp pole) to 3 (climbs to the top readily with no issues).

The potential prophylactic antidote, AuTS, was injected intraperitoneally (ip) into mice (3−6 animals) at 50 mg/kg, 5 min before the exposure to phosphine. Control animals received phosphine alone. In addition, the putative antidote (AuTS, 50 mg/kg) was given 1 min after the exposure of the mice to phosphine.

Respirometric Experiments. An Oxygraph O2k Polarographic instrument (Oroboros Instruments, Innsbruck, Austria), equipped with a Clark-type electrode for high-resolution respirometry, was used to measure oxygen flux. Mitochondrial buffer, MiR05,[27] (2.1 mL) was added to both chambers of the respirometer and allowed to equilibrate for 20 min at 25 °C before ∼100 μL of the mitochondrial particles (prepared as above) was added. Mitochondrial respiration was then observed with the addition of cytochrome *c* (final concentration 10 μM), succinate (final concentration 10 mM), and rotenone (to prevent back reaction through complex I, final concentration 0.5 μM). Phosphine was added in 25−100 μL increments to the respiring mitochondria particles from a saturated phosphine solution prepared by adding deoxygenated phosphate buffer to AlP in a septum-capped vial with minimal headspace.[28]

Protein Isolations and Enzyme Assay. Cytochrome *c* oxidase was prepared as previously described[29] from intact bovine heart mitochondria using a modified Harzell−Beinert procedure (without the preparation of Keilin−Hartree particles). The enzyme was determined to be spectroscopically pure if the 444 to 424 nm ratio for the reduced enzyme was 2.2 or higher.[30] Enzyme concentrations were determined as total heme *a* using the differential (absorption) extinction coefficient of $\Delta\varepsilon_{604}$ = 12 $mM^{-1}\cdot cm^{-1}$ for the reduced minus oxidized spectra of the mammalian and bacterial enzymes, respectively.[31] Concentrations throughout are given on a per enzyme concentration basis (i.e., [heme *a*]/2).

Steady-state kinetics were performed with the isolated enzyme as described by Nicholls et al.[32] The concentration changes of the electron donor, bovine ferrocytochrome *c*, were monitored through its absorbance at 550 nm (minus 540 nm, an isosbestic point in the spectrum of cytochrome *c*) in the presence of excess sodium ascorbate (14.5 mM) in normoxic solution, 0.1 M potassium phosphate, pH

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7.44, 0.02% laural maltoside (Anatrace). The fractional oxidase activity, $[E]$, was determined by the following equation

$$[E] = [c^{2+}]_0 \cdot [c^{3+}]_t / [c^{3+}]_0 \cdot [c^{2+}]_t \quad (1)$$

where $[c^{2+}]_0$ = fraction at time 0, $[c^{3+}]_t$ = fraction at time t, $[c^{3+}]_0$ = fraction at time 0, and $[c^{2+}]_t$ = fraction at time t.

Statistical Analysis. Data was analyzed using Kaleidagraph software. A p-value ≤ 0.05 was considered significant.

RESULTS

We have previously shown that *G. mellonella* caterpillars provide a reasonable model for screening antidotes to cytochrome *c* oxidase toxicants, such as cyanide.[24] Oxygen consumption was initiated by adding cytochrome c (to replace any lost when the mitochondria were lysed) along with the electron donor succinate, and rotenone (complex I inhibitor) to prevent backflow. The respirometric inhibition (decreased oxygen flux, JO_2) of *G. mellonella* mitochondrial particles[24] observed was quite linear (R^2 = 0.95) with respect to micromolar phosphine additions as shown in Figure 1.

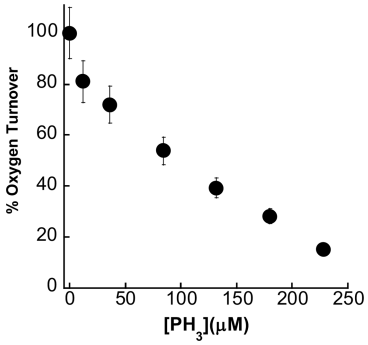

Figure 1. Respirometric response of *G. mellonella* mitochondrial particles titrated with PH_3. Oxygen consumption was assessed in *G. mellonella* mitochondrial particles diluted in MiR05 respirometric solution (see Experimental Section for details). The tissue suspension (2.1 mL) was allowed to equilibrate in chamber for ~10 min prior to measuring oxygen consumption. Default respirometric settings of block temperature, 25 °C; stir bar speed, 400 rpm; and data recording, 2 s were used. All reagents/substrates quanitations are given as final concentrations. Cytochrome *c* (10 μM), succinate (0.5 mM), and rotenone (0.5 μM) were added to the 2.1 mL of respirometric solution containing 100 μL of mitochondrial particles and the oxygen flux recorded over 5 min (JO_2 of ~140 pmol/s · mL). Subsequently, PH_3 was added, from a saturated solution, resulting in PH_3 concentrations of 12–230 μM in the respirometer, and the oxygen flux was followed until it was constant (~5 min).

Exposure of *G. mellonella* larvae to phosphine in a closed container for 20 min caused ~50% of the larvae to become immobile (paralyzed), and the time (recovery time) until the paralyzed larvae regained their ability to move was then recorded. Any larvae that were not immobilized were recorded as having a recovery time of zero. A dose–response of this adjusted recovery time of the *G. mellonella* larvae exposed to phosphine (12–10 000 ppm·min) was subsequently determined (see Figure 2). An exposure of 4300 (±700) ppm·min phosphine (over a 20 min time span) induced a state of paralysis that lasted ~35 min, a conveniently repeatable response.

Once a reproducible recovery time for the larvae was obtained, putative antidotes at levels that showed no visible toxicity to the *G. mellonella* larvae (AuTS, 25 mg/kg; AuTM, 1 g/kg; AuTG, 1 g/kg) were administered by injection into the most distal left abdominal proleg 10 min prior to exposure to phosphine gas. All the gold complexes tested significantly decreased the mean time until the larvae were recovered (AuTM, 3 (±3) min; AuTG, 0 min; AuTS, 2.1 (±0.7) min; Figure 3A) and decreased the median time to be zero for all the antidotes when used prophylactically. More impressively, when the antidotes were given to the larvae at 10 min after exposure to phosphine, all proved to also be effective when given therapeutically (mean times until recovery: AuTM, 19 (±6) min; AuTG, 17 (±6) min; AuTS, 15(±4) min; median times until recovery: AuTM, 3 min; AuTG, 0 min; AuTS, 0 min; Figure 3B).

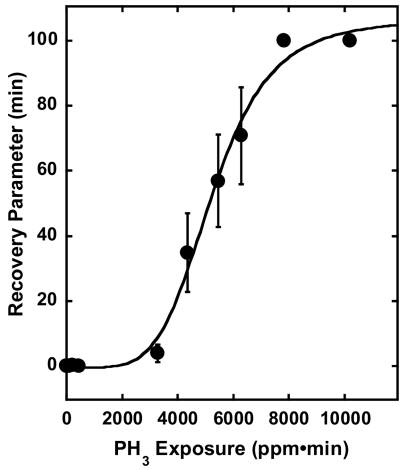

Figure 2. Dose–response data for *G. mellonella* larvae exposed to varying amounts of PH_3. The larvae were exposed to PH_3 gas generated by the reaction of Ca_3P_2 with 20 mL of 1.2 M H_2SO_4 in a closed vessel (see Experimental Section). In sham controls, the addition of the same amount of acid to a reaction vessel (separated from the larvae) caused no change in the larval behavior. The time from which each larva ceased movement until movement began again (recovery time) was recorded. Each group of larvae (~10) were exposed to PH_3 at 12–10 000 ppm·min. Roughly 60% of control larvae were incapacitated, and any larvae that did not knockdown were assigned recovery times of 0 min. The few larvae that had recovery times over 120 min were not scored.

Since it is difficult to isolate cytochrome *c* oxidase from *G. mellonella* larvae in large enough amounts for steady-state turnover experiments and it has been shown that minimal differences between enzymes isolated from different species exist, the turnover experiments were performed with enzyme isolated from bovine hearts. The steady-state turnover of oxygen by bovine cytochrome *c* oxidase was monitored by following the absorbance changes of cytochrome *c*, the oxidase electron donor, after providing a source of electrons for cytochrome *c*, sodium ascorbate (Figure 4A, open circles). Once the steady state was established, phosphine (to 100 μM) was added (at ~100 s), resulting in inhibition of the enzyme. The resulting time course of the inhibition of cytochrome *c* oxidase was fit by a single exponential: $[E]_{active} = 0.87e^{-0.033t} + 0.13$ (Figure 4B). The inactivation rate was proportional to the phosphine concentration with a k_{on} calculated to be 3.3×10^3 $M^{-1} \cdot s^{-1}$ with 13% of the enzyme still active at the phosphine concentration of 100 μM. This residual activity is proportional to the apparent K_i, which was determined to be 13 μM, similar to those previously reported.[33] To test if the observed amelioration of phosphine toxicity by gold(I) complexes

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Chem. Res. Toxicol. 2019, 32, 1310–1316

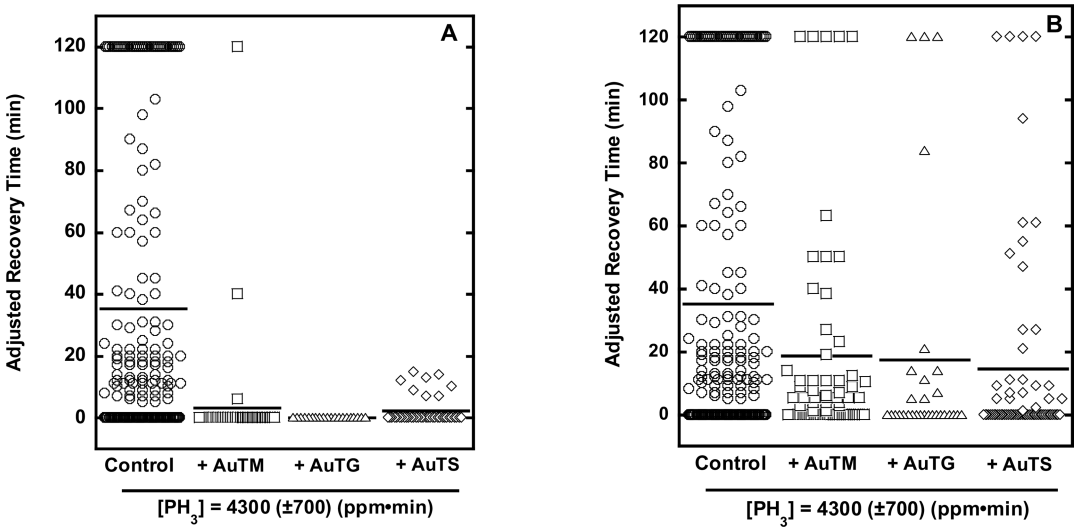

Figure 3. Prophylactic and therapeutic use of sodium aurothiomalate (AuTM), aurothioglucose (AuTG), and auro-sodium bisthiosulfate hydrate (AuTS) against phosphine toxicity in *G. mellonella* larvae. Larvae were injected with each of the three putative antidotes (AuTM, 1 g/kg; AuTG, 1 g/kg; AuTS, 25 mg/kg) either 5 min prior (A) or 10 min after (B) a PH_3 exposure of 4300 (±700) ppm·min. The mean time until recovery was measured for each putative antidote vs control. Roughly 60% of control larvae were incapacitated after PH_3 exposure. Any larvae that did not knockdown were assigned recovery times of 0 min. The few larvae that had recovery times over 2 h were scored as 120 min. In panel A (prophylactic testing), for each gold compound tested, $p < 0.005$ when compared to control; in panel B (therapeutic testing), for each gold compound tested, $p < 0.01$ when compared to controls. The mean for each group is shown as a bar. The median recovery times were 0 in all cases, except for controls and the therapeutic administration of AuTM, which resulted in a median recovery time of 3 min.

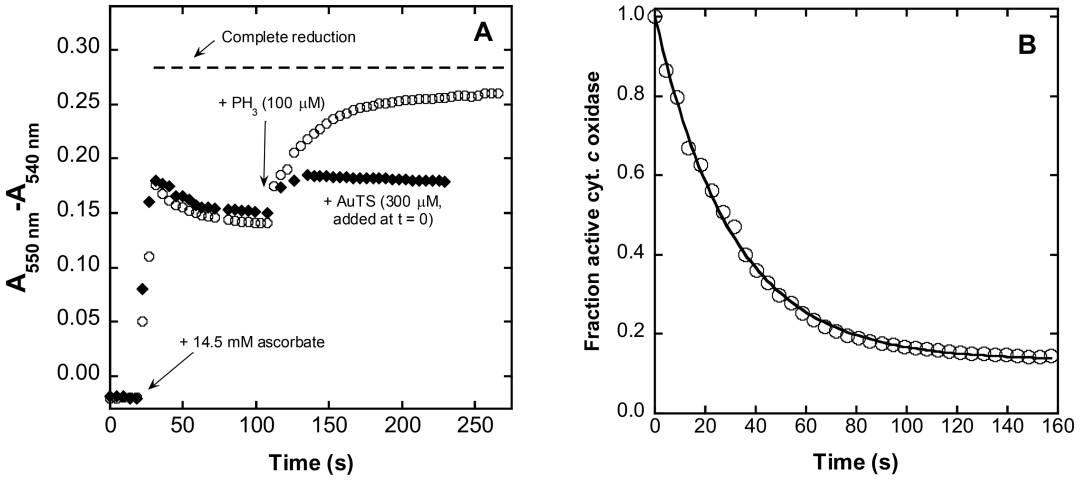

Figure 4. Cytochrome *c* oxidase steady-state turnover: inhibition by phosphine and rescue by auro-sodium bisthiosulfate hydrate (AuTS). Cytochrome *c* oxidase (0.194 μM) turnover was followed by observing the oxidation of reduced cytochrome *c* (14 μM) monitored spectroscopically at $A_{550\ nm} - A_{540\ nm}$ (T = 25 °C, 0.1 M sodium phosphate, pH 7.44, 0.02% lauryl maltoside) over time. Turnover was initiated by the addition of 1.45 mM sodium ascorbate. (A) Representative plots for the addition of phosphine (100 μM, open circles) to the active enzyme. Addition of 300 mM AuTS prior to ascorbate initiation of turnover (closed diamonds, symbols have been slightly offset so as to view both data sets) and subsequent addition of 100 μM phosphine. (B) A single exponential fit (solid line) to the fraction of active enzyme vs time (open circles) calculated according to eq 1 (see Experimental Section). All reagents/quantities are given as final concentrations.

(Figure 3) could indeed be attributable to antagonism of cytochrome *c* oxidase activity, AuTS (300 μM) was added to the enzyme solution prior to the initiation of steady-state conditions by addition of ascorbate (Figure 4A, closed diamonds). When phosphine (100 μM) was subsequently added, the steady-state turnover was roughly 70% of that observed for the normally functioning enzyme.

After screening the potential antidotes in *G. mellonella* larvae, a preliminary set of similar experiments were then carried out in mice. Swiss-Webster mice were exposed to phosphine gas, produced by the same method as used with the *G. mellonella* larvae, in a closed container for 15 min. This dose of phosphine, 3200 (±500) ppm·min, did not cause the mice to "knockdown" but did induce a severely lethargic state (motionless in open field). The mice were then examined by a pole-climbing behavioral assessment (see Experimental Section for details). Mice were examined 5 days prior to the phosphine experiments in order to obtain a baseline and then subjected to the pole test starting immediately following their exposure to phosphine. Mice given the AuTS antidote (50 mg/kg, previously determined to cause no change in behavior by pole testing and chosen based on the mean recovery time in *G. mellonella*) 5 min prior to the phosphine exposure performed as well as control mice that had never been exposed to

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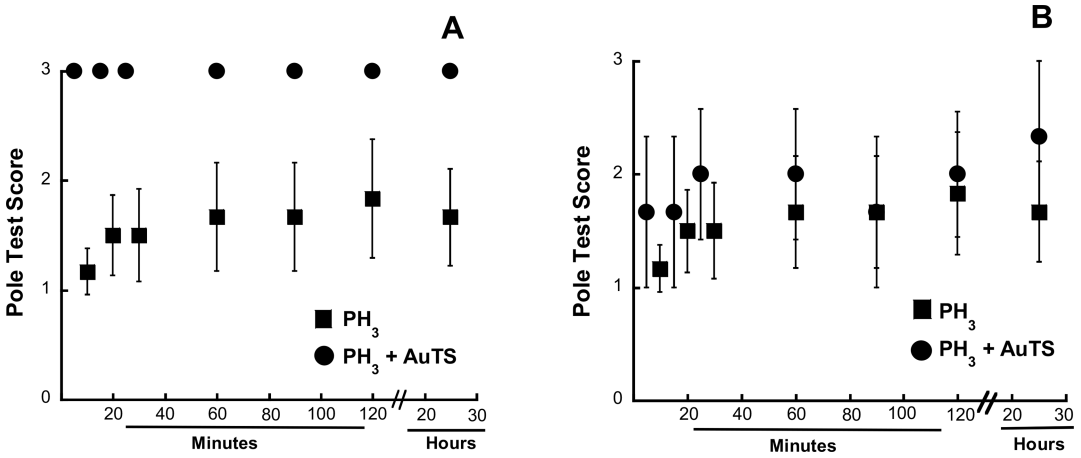

Figure 5. Prophylactic and therapeutic use of aurobisthiosulfate (AuTS) in PH_3 exposed mice. Mice were given 50 mg/kg of AuTS intraperitoneally (closed circles) either 5 min before (A) or 1 min after (B) a PH_3 (closed squares) exposure of 3200 (±500) ppm·min (over a 15 min time period), The mice were examined using a behavioral assessment (pole-climbing test, see Experimental Section) to evaluate their response to the toxicant and putative antidote (AuTS).

phosphine (Figure 5A). However, when mice were exposed to phosphine and given the AuTS antidote 1 min after the toxicant exposure, the results of the pole test were less impressive with only a very modest improvement in performance (Figure 5B).

DISCUSSION

It is intrinsically clear from the data (Figures 3A & B) that, in the caterpillars, all three of the Au(I) compounds are highly effective antidotes for phosphine. That is, the original concept has been at least circumstantially validated in a biological system; Au(I) complexes are indeed able to detoxify phosphine, presumably through scavenging (coordination) and decorporation. Regarding the trials with mice, the compounds are certainly prophylactically effective (Figure 5A) but when administered after the toxicant, any beneficial effects were much more modest in these assays (Figure 5B). This result should not, however, be taken to indicate that that the approach will ultimately prove to be of no therapeutic application for two main reasons. First, it is presently unclear exactly why Au(I) complexes seem to be significantly therapeutic in the caterpillars (Figure 3B) but not the mice (Figure 5B). The compounds may have significantly different pharmacodynamic/kinetic characteristics in the two organisms, in which case, there may be related structures exhibiting such properties more suitable for therapeutic use in mammals. At this juncture, especially given that there is no antidote for phosphine currently available, any detectable ameliorative effect is encouraging. Second, most human victims reach the clinic having ingested a phosphide salt, and the exposure is ongoing as the phosphide continues to release phosphine gas through hydrolysis in the stomach. Additionally, while phosphides are employed as fumigants in western countries, for indoor control of insects and outdoor control of rodents, it is not clear that their use is so effectively regulated worldwide. The recent increase in the application of drones to crop-dusting operations, particularly in Asia, could conceivably lead to future exposures of larger human populations, either through accident or with malicious intent, not to mention the possibility of release by deliberate detonations. Any individuals thus exposed to particulate phosphides dispersed in air will likely have infiltration of phosphide particles into the esophagus and airways, also adhered to clothing. In all such cases, where slow and continuing release of phosphine gas is to be expected, the availability of effective prophylactics to prevent any further toxic dose exacerbating the condition of the victims could have life-saving consequences.

The mechanism(s) through which phosphine exerts its toxicity is (are) seemingly complicated[13,34,35] and remain(s) incompletely delineated.[36−38] We think it pertinent to consider if the present findings shed any light on these matters. For almost half a century, mitochondria have been[17] and continue to be[39] identified as key targets for disruption by phosphine through inhibition of cytochrome *c* oxidase.[33,40−43] In response to sublethal phosphine exposure, the *G. mellonella* larvae used in the current study exhibit dose-dependent (Figure 2) temporary paralysis (knockdown), from which they appear to fully recover. This behavior is analogous to that obtained employing the bona fide cytochrome *c* oxidase inhibitors azide, cyanide, and sulfide.[24] Consequently, while we have not set out to examine this particular question rigorously, our observations concerning the caterpillars do appear to be at least consistent with a mechanism of acute phosphine toxicity primarily involving inhibition of cytochrome *c* oxidase. If this is so, then it follows that the different response to the antidotes of phosphine-challenged mice (Figure 5) compared to the caterpillars (Figure 3) is plausibly due to another toxic mechanism operating in the mice, which might not involve reversible inhibition of cytochrome *c* oxidase.

In mammals, acute phosphine/phosphide poisoning is reported to lead to death by cardiopulmonary failure, with microscopically visible injury to myocardial tissue.[35,44] This shares some similarity with acute cyanide and sulfide toxicity in mammals, where death is also the result of cardiopulmonary collapse, but cyanide and sulfide act more rapidly[45,46] and principally on the central nervous system stimulating cardiopulmonary function.[47,48] The measured inhibition constant (K_i = 13 μM) and on-rate (k_{on} = 3.3 × 10^3 $M^{-1}\cdot s^{-1}$) for phosphine reacting with isolated cytochrome *c* oxidase (Figure 4) are, respectively, 2 orders of magnitude greater and 3 orders of magnitude slower than the corresponding reaction of the enzyme with cyanide,[49] in keeping with the less toxic nature of phosphine compared to cyanide. It follows that lethal doses of inhaled phosphine may require prolonged exposure as

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Chem. Res. Toxicol. 2019, 32, 1310−1316

recently reported,[50−52] but there remains an observable difference between the behavior of phosphine and the better characterized mitochondrial toxicants in mammals, again suggesting that there could be at least one other toxic mechanism in play, possibly nonmitochondrial. Rahimi et al.[53] have recently shown that phosphine poisoning in rats can be ameliorated through blood transfusion, clearly implicating some component of the blood/vasculature as a target for the toxicant. This finding seems to be in keeping with earlier observations[54,55] that hemolysis and methemoglobinemia may correlate with severity of outcome in aluminum-phosphide-poisoned human patients. There is a paucity of information regarding the reaction of phosphine with hemoglobin and red blood cells, the available literature being more than 25 years old.[56,57] Further effort in this area now appears to be warranted.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.9b00095.

Raw data for *G. mellonella* and mouse phosphine toxicity along with antidote amelioration for the experiments presented in Figures 2, 3, and 5 (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline

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