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Complete List of Authors:	Wataha, John; University of Washington, Restorative Dentistry Hobbs, David; Savannah River National Laboratories Lockwood, Petra; Medical College of Georgia, Oral Biology and Maxillofacial Pathology Davis, Ryan; Medical College of Georgia, School of Dentistry Elvington, Mark; Savannah River National Laboratories Lewis, Jill; Medical College of Georgia, Oral Biology & Maxillofacial Pathology Messer, Regina; Medical College of Georgia, Oral Biology and Maxillofacial Pathology
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Peroxotitanates for biodelivery of metals

John C. Wataha[1]*, David T. Hobbs[2], Petra E. Lockwood[3], Ryan R. Davis[3], Mark C. Elvington[2], Jill B. Lewis[3], Regina L.W. Messer[3]

[1]*Department of Restorative Dentistry, University of Washington, Seattle, WA 98026 USA*

[2]*Savannah River National Laboratory, Aiken, SC 29801 USA*

[3]*Department of Oral Biology, Medical College of Georgia School of Dentistry, Augusta, GA 30912 USA*

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*Corresponding author:

John C. Wataha, DMD, PhD
Department of Restorative Dentistry
D770B HSC, Box 357456
University of Washington
1959 NE Pacific St.
Seattle, WA 98196-7456

Phone: (206) 543-5948
Fax: (206) 543-7783
E-mail: jwataha@u.washington.edu

Abstract

Metal-based drugs are largely undeveloped in pharmacology. One limiting factor is the systemic toxicity of metal-based compounds. A solid-phase, sequestratable delivery agent for local delivery of metals could reduce systemic toxicity, facilitating new drug development in this nascent area. Amorphous peroxotitanates (APT) are ion exchange materials with high affinity for several heavy metal ions, and have been proposed to deliver or sequester metal ions in biological contexts. In the current study, we tested a hypothesis that APT are able to deliver metals or metal compounds to cells. We exposed fibroblasts (L929) or monocytes (THP1) to metal-APT materials for 72 h in vitro, then measured cellular mitochondrial activity (SDH-MTT method) to assess the biological impact of the metal-APT materials *vs.* metals or APT alone. APT alone did not significantly affect cellular mitochondrial activity, but all metal-APT materials suppressed the mitochondrial activity of fibroblasts (by 30-65% of controls). The concentration of metal-APT materials required to suppress cellular mitochondrial activity was below that required for metals alone, suggesting that simple extracellular release of the metals from the metal-APT materials was not the primary mechanism of mitochondrial suppression. In contrast to fibroblasts, no metal-APT material had a measurable effect on THP1 monocyte mitochondrial activity, despite potent suppression by metals alone. This latter result suggested that 'biodelivery' by metal-APT materials may be cell type-specific. Therefore, it appears that APT are plausible solid phase delivery agents of metals or metal compounds to some types of cells for potential therapeutic effect.

Key words: drug delivery, heavy metals, cytotoxicity, mitochondrial activity, monocyte

1. Introduction

The distinctive oxidation-reduction properties of metal ions, combined with their coordination chemistries, allow them to serve critical roles in biological structure, catalysis, and transport. These properties also make them attractive candidates for novel drugs in medicine, yet because of their relatively potent systemic toxicity, only a few metal-based drugs are currently used, most notably Au(I)-based drugs to treat rheumatoid arthritis and cis-platinum compounds to treat certain cancers. Even with the success realized with these Au and Pt-based drugs, systemic toxicity of these metal-based drugs has severely limited development of other metal-based therapeutics. One promising strategy to mitigate the systemic toxicity of metal-based drugs is local delivery of metal ions or metal-based compounds. In the current study, we extend our ongoing work into the suitability of titanate compounds as agents for local delivery of metal ions or compounds to cells.

Monosodium titanates (MST, $NaTi_2O_5H \cdot xH_2O$) are amorphous metal oxides that strongly bind strontium and the actinides (e.g. plutonium, neptunium, and uranium), and this affinity has been employed to sequester ionic radionucides from nuclear waste solutions into a solid phase [1,2]. A new class of titanate materials, referred to as amorphous peroxotitanates (APT, $H_vNa_wTi_2O_5 \cdot xH_2O[yH_zO_2]$, $v + w = 2$ and $z = 0$-2), have recently been synthesized by converting the Ti-OH groups in the MST to Ti-O-OH [3-4]; APT have metal sorption characteristics that are similar to MST, but have more favorable sorption kinetics [3,4]. The titanium-peroxo species imparts a bright yellow color to the APT powder that is retained in aqueous suspension (Fig. 1). MST and APT are both synthesized as particulates ranging in size from approximately 0.1 to 10 μm ([5], Fig. 1).

In addition to strontium and the actinides, MST and APT have high adsorption capacities for Cd(II), Hg(II) and Au(III) [5]. For example, MST has an adsorption capacity of 1.8 μmol-Cd(II)/μg of MST when 'loaded' from water, somewhat less (0.75 μmol/μg) from phosphate

buffered saline [5]. Hg(II) and Au(III) also exhibit substantial adsorption capacities (0.90 and 0.45 μmol/μg in water, respectively) that are dependent on the ionic strength of the loading solution. The adsorption capacities of APT are similar to MST, but the kinetics of APT adsorption are faster [3-4], suggesting better efficiency where rapid ion exchange is desirable, for example in continuous solid phase separations.

The biological properties of MST and APT have been explored only *in vitro*, but both appear to have properties favorable for further development for sequestration or delivery of metals in biological environments [6]. By themselves, MST and APT particles at concentrations up to 100 μg/mL have few overt effects on the metabolic activity of monocytes or fibroblasts. Studies estimating cellular metabolic response have been restricted to mitochondrial activity, however [5,6]. Neither APT or MST appear to activate monocytes to secrete inflammatory mediators despite their particulate nature and small size [6]. Yet, all work in this area is preliminary and needs further investigation.

Recently, our group exposed cells to MST and APT loaded with two gold compounds. Because both Au(III) and Auranofin® (a Au(I)-organic compound, [7]) suppress mitochondrial succinate dehydrogenase (SDH) activity at relatively low (μM) concentrations, we hypothesized that APT or MST combined with gold compounds also might expose cells to the gold compounds sufficiently to suppress SDH activity. Such suppression by gold-MST/ATP materials was observed [8] and suggests a delivery of gold compounds to the cells, because titanates alone had no measurable effect on SDH activity. Our results further suggested that APT was more effective at such 'biodelivery', that not all cell types respond similarly, and that Au(III) and Auranofin® differ in the efficiency of the delivery [8].

The biological effects of gadolinium, palladium, platinum, and mercury compounds have been studied extensively (e.g., [9-14]). Biological concerns about mercury center around its use in dental amalgam, its neurotoxicity, and a significant environmental impact [15-18]. Cisplatin is

a successful anti-neoplastic drug and because of its success in this application, the biological effects of many palladium- and platinum-organic compounds have been investigated in search of other anti-cancer agents (e.g., [19]). Gadolinium compounds are well known radiographic imaging/contrast agents in medicine [20-23]. We selected these metals for study with titanates because of their biological relevance.

Our current work tests a hypothesis that APT can deliver Pt, Au, and Hg compounds and ions to two types of cells. We first established the potency of the metals alone as suppressors of cellular metabolic (SDH) activity of two cell types, then related the potency of metal-APT materials to the nature of the metal and its loading efficiency onto APT. Our results here support further exploration of metal-titanate complexes as novel drug delivery agents for metals or metal compounds to cells from a solid phase that is able to be delivered and sequestered locally. Such a strategy may permit delivery of relatively high local concentrations of metals with less systemic toxicity.

2. Materials and methods

2.1 APT Loading, Assessment of Loading

Palladium, gadolinium, mercury, and platinum compounds were investigated in the current study (Table 1). We focused on amorphous peroxotitanates (APT) vs. monosodium titanates (MST) because the former exhibited faster sorption kinetics for exchange with metal species such as gold [3,4,6,8]. APT was synthesized from monosodium titanate (Optima Chemical Group, LLC Douglas GA) as described previously [8].

Metal-APT materials for palladium, mercury and platinum compounds were prepared by combining 0.25 g of APT suspended in 1.4 g of water having a pH of 6.9, with 10 mL of a phosphate buffer saline (PBS) solution containing the desired metal/metal compound (Table 1) at ambient temperature for a minimum of 48 h. The gadolinium-APT material was prepared by adding the APT suspension to a pH 3 solution of gadolinium. The metal-APT materials were separated from the parent solutions by centrifugation (RCF = 1200 x *g*) for 3 min. The solid phases were rinsed quickly with 6 portions of chilled PBS (4° C; pH = 7.4) and stored as moist solids with water contents of about 75 wt%. The Gd-APT sample was first rinsed with 3 portions of dilute nitric acid solution (pH 3) followed by 3 portions of the PBS solution. Complete drying of the metal-loaded APT materials was avoided because resuspension of the dried metal-loaded APT materials proved difficult in cell-culture solutions. The quantity of metal loaded onto the APT loading was determined by analyzing the complexes for metal content by the difference in metal concentrations in the solution before and after contact with the APT solids using inductively coupled plasma emission spectroscopy (ICP-ES) for all metals except mercury, which was measured using cold vapor atomic absorption spectroscopy (CV-AA). Metal loading efficiency was reported as the number of femtomoles (fmol) of each metal per μg of APT.

2.2 Exposure of cells to metal compounds alone or metal-APT materials

In these experiments, we focused on monocytes because of their prominent role in orchestrating inflammatory responses and role in chronic inflammatory disease, and fibroblasts to assess possible applications of APT in connective tissues. THP1 monocytes (ATCC TIB202, American Type Culture Collection, Manassas VA) were cultured in suspension in RPMI 1640 cell-culture medium (InVitrogen-Gibco, Carlsbad CA) and 10% fetal bovine serum, with 2 mmol/L glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin, all at pH 7.2 (Invitrogen-Gibco). L929 fibroblasts were cultured in anchorage dependent format in Ham's F12 cell-culture medium with 5% fetal bovine serum and glutamine, penicillin, and streptomycin, as with THP1 monocytes.

Cells (250,000 cells/mL for THP1; 5000 cells/cm^2 for L929, both in 200 μL) were plated in 96-well format (n = 8, flat-bottom format for L929, round-bottom format for THP1). For experiments in which the metal compounds were used alone, stock concentrations of metal compounds (Table 1) were diluted using sterile PBS and aseptic techniques to prepare a series of solutions which would provide a range of concentrations of the metal species when 20 μL was added to 200 μL of the cell-culture. Controls contained no added metal species. For some metal species (Hg(II), Pt(IV), cis(Pt), the initial concentration range that was selected was too cytotoxic and experiments were adjusted using lower ranges of concentrations of metal species. Cells were incubated for 72 h at 37° C and 5% CO2 in 100% relative humidity.

For experiments where APT or metal-APT materials were added, aqueous stock solutions containing 3000 mg/L of the APT or metal-APT sample were prepared aseptically in sterile PBS, then diluted to a series of secondary solutions that would provide final APT concentrations of 1.0-100.0 μg/mL (n = 8/condition). Controls contained APT alone or no APT. As with the THP1, L929 fibroblasts were incubated for 72 h at 37° C and 5% CO_2 in 100% relative humidity.

2.3 SDH assay

After 72 h, cell response to metals alone, APT alone, or metal-APT combinations was

assessed by measuring mitochondrial succinate dehydrogenase (SDH) activity using the MTT method (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Sigma-Aldrich). To measure SDH activity specifically, the MTT was added to cultures in a balanced salt solution containing only disodium succinate as a source of electrons for MTT reduction. Details of this procedure, adjusted for use with titanate suspensions, has been reported previously [5,6]. The assay was performed in a solution of 2 molal sodium succinate to focus the assay on mitochondrial function. This focus has been reported as a reasonable overall estimate of cellular metabolic function [23]. SDH activity was normalized to controls without metal or APT addition, then plotted as a function of metal-APT or metal concentration. The concentration required to suppress SDH activity by 50% in metal-alone experiments was determined graphically. For metal-APT experiments, ANOVA and Tukey post-hoc analyses were used to compare the effects of different metal-APT concentrations ($\alpha = 0.05$).

3. Results

3.1 Metal-APT loading

All metal species in Table 1 were successfully loaded to some degree onto the amorphous peroxotitanate (APT) material (Table 2). The degree of metal loading ranged from a high for Pd(II) at 142 fmol/μg of APT to a low for Pt(IV) at 18 fmol/μg. Gd(III), Hg(II), and cisPt loaded at intermediate levels of 78.8, 37.8, and 56.8 fmol/μg-APT, respectively. The degree of adsorption of a metal onto the APT depended on a number of factors including the metal concentration in solution, the metal species in solution, the inherent specificity for adsorption of the metal ion or compound and the phase ratio used (i.e., ratio of solution volume to mass of APT). In this study we made no attempt to maximize the loading of a metal onto the APT. For the syntheses of the metal-loaded APT materials used in this study, we kept the quantity of APT and phase ratio of metal solution and APT constant. Thus, the metal-APT loadings reflected both the effects of the initial metal concentration in solution and the specificity of the APT material for a specific metal or metal compound. For example, about twice as much Gd(III) loaded onto the APT as Hg(II) even though the initial Hg(II) concentration was about 70% higher than that of the Gd(III). The higher loading of cisPt onto APT compared to Pt(IV) suggested that the form of the metal played an important role in the affinity of the APT to load a particular metal.

3.2 Cellular response to metal compounds alone

Each of the metal species suppressed succinate dehydrogenase (SDH) activity in a dose-dependent manner (Figs. 2,3). However the potency of metal-induced SDH suppression varied widely among the metal species. For example, a concentration 200-300 μM of Gd(III) suppressed the SDH activity of THP1 monocytes by 50% vs. no-metal controls ($TC50_{SDH}$), yet the $TC50_{SDH}$ of cisPt was < 1 μM. The graphically determined $TC50_{SDH}$ values for other metal species fell between these extremes (Table 3). For some metal species such as Gd(III) or

Pd(II), there were distinct differences in how THP1 monocytes and L929 fibroblasts responded. For example the $TC50_{SDH}$ for THP1 monocytes to Pd(II) was > 300 μM, whereas for L929 fibroblasts this value was < 100 μM. Other metal species such as cisPt, Pt(IV), and Hg(II) had similar potencies to both cell types. Overall, SDH suppression induced by these metal species appeared sufficient to use it as a measure of biodelivery of peroxotitanates loaded with these species.

3.3 Cellular response to metal-APT materials

For THP1 monocytes there was no evidence of significant SDH suppression by metal-APT materials. Even at the highest loaded APT concentration (100 μg/mL), no statistically significant mitochondrial suppression by metal-APT materials was observed (Fig. 4). A tendency of Pd(II)-APT materials to increase SDH activity was suggested, but not confirmed statistically at 100 μg/mL. On the other hand, the SDH activity of L929 fibroblasts were significantly suppressed by all metal-APT materials in a dose-dependent fashion (Fig. 5). At the highest concentration of APT complexes (100 μg/mL), SDH suppression ranged from 30% (Pd(II)) to 65% (cisPt) (Table 3). This suppression strongly suggested that the metal-APT materials were able to 'deliver' the metal species in some fashion to affect cell function.

Note that for all metal species except cisPt, metal-APT materials-induced suppression of SDH activity occurred at metal concentrations far below the $TC50_{SDH}$ values required for metal species alone (Table 3). For example, >500 μM of Gd(III) were required to suppress L929 SDH activity by 50%, yet a maximum metal delivery of only 7.9 μM for Gd(III)-APT suppressed SDH activity by 45%. These results suggested that the effect of the loaded APT material was not simply occurring via extracellular release of the metal species from the loaded material with subsequent cellular interaction. For cisPt, however, the cisPt-APT material seemed to impede SDH suppression of cisPt alone (Table 3).

4. Discussion

The primary hypothesis of the current study was that gadolinium, mercury, palladium, or platinum compounds could be 'biodelivered' to cells via metal-APT materials. Testing this hypothesis required that we determine the dose-response of cells to metal ions alone and identify concentrations that altered cell activity. As a measure of cell activity, we chose mitochondrial function (SDH) because it is simpler to measure than many other cell processes, yet is central to the appropriate function of the cell. In the context of the current study, the $TC50_{SDH}$ values of Pd(II), Pt(IV), and Hg(II) in Table 3 were in reasonable agreement with those reported previously for other cells and conditions (e.g., [24-28]), giving consideration to the many variables that influence such studies. The ability of these metals to suppress mitochondrial activity at low (micromolar) concentrations, combined with their affinity for APT made them good candidates to assess the biological effects of metal-APT materials vs. APT alone. However, many other endpoints in cell function could be and should be tested in future work that assesses the effects of APT materials with or without metal loading.

The current data extend our previous reports using the Au(I)-organic complex Auranofin® [8], and support the possibility of loading APT with other metal ions or metal compounds (Table 2), although the chemical nature of the loaded compound deserves further investigation. The concentrations of solutions used to 'load' APT were not the same for all metal compounds, ranging from 6,100 μM for cisPt to 27,400 μM for Pd(II) (Table 1). These different loading concentrations resulted from the upper limits of aqueous solubilities of the compounds in PBS. To maximize our chances of demonstrating metal delivery to cells, our strategy was to prepare metal-loaded APT materials with metal loaded as high as possible; thus, we used the loading solutions in Table 1.

The difference in loading efficiency between Pt(IV) and cisPt in Table 2 suggests that loading might be regulated or optimized by the nature of the coordinating ligands and oxidation

state of a metal ion. Pt(IV) compounds are kinetically inert, whereas Pt(II) compounds are much more reactive [29]. Because cisPt features platinum in the +2 oxidation state, reaction of cisPt with the peroxotitanate would be expected to be more rapid and therefore proceed to a further extent at 48 h than with the Pt(IV). Thus, the Pt(II) loading onto the APT sorbent was approximately a factor of 3 higher than that of Pt(IV) in spite of the fact that the solution concentration of Pt(IV) was 2.5 times higher than that of the cisPt (Table 2).

Suppression of L929 fibroblast SDH activity by metal-APT materials strongly suggested that the materials were able to 'deliver' the metals to the fibroblasts (Fig. 5). All metal-APT materials exhibited some ability to suppress mitochondrial activity. Yet, this ability was not strictly dependent on the degree of metal loading (Tables 2,3). For example, the Pd-APT materials, which contained the most metal at 142 fmol/μg, suppressed L929 mitochondrial activity the least (30%) among the metals tested. Pt(IV)-APT, which at 18 fmol/μg contained almost a factor of 8 times lower metal loading than Pd(II)-APT, suppressed mitochondrial activity more than Pd(II)-APT. Scrutiny of Tables 2 and 3 also does not support an inverse relationship between loading efficiency and ability to affect cellular changes. Collectively, these observations suggest more complex metal-APT interactions with cells.

With the exception of cisPt, the metal-APT materials exhibited a greater ability to suppress L929 mitochondrial activity than would have been predicted from dose-response curves for the metals alone. For example, > 500 μM of Gd(III) was required to suppress L929 mitochondrial activity by 50% (Table III). If all of the Gd from the APT-Gd complex was released, the Gd concentration would be only 7.9 μM, 60-fold below the TC50 concentration. Yet, the Gd-APT material suppressed mitochondrial activity by 45%. This trend was true for all metal-APT materials except cisPt. In this latter case, the cisPt-APT was a less potent suppressor of activity than predicted by loading.

The current study focused on the question of whether delivery of metal ions to cells is

possible from metal-APT complexes; given data supporting such biodelivery in fibroblasts (Fig. 5), future study into the mechanisms of such delivery is strongly suggested. At first glance, several mechanisms are consistent with our current data (Fig. 6). Metal-APT materials could have simply released the metals extracellularly with the metal ions subsequently suppressing fibroblast SDH activity. We suggest that this mechanism, although quite plausible, was not the major mechanism in force in the current experiments. As discussed above, the amount of metal maximally carried by the APT was insufficient to cause the observed SDH suppression in fibroblasts, even if 100% of the metal were released extracellularly (Table 3, Figs. 2,5). Although we have not yet assessed metal release for Pt, Pd, or Gd compounds, in past pilot studies, we have assessed desorption from Au(III)-APT and AF-APT complexes from 2 h to 21 days. No Au release was detected at pH 7.4, the pH of the cell-culture medium, to the level of detection of 1 mg/L (ICP-ES). Collectively these data tentatively imply that the mechanism of L929 mitochondrial suppression in the current experiments may not have simply been a function of metal released extracellularly from the metal-APT complex. Rather, our results indicate that delivery of the metal involves some form of cell-APT complex interaction (Fig. 6), or a combination of extracellular release and cell-APT interactions. Yet, a role for desorbed metals as contributors to the SDH suppression observed in Fig. 5 should not be ignored and given our demonstration of some sort of delivery to fibroblasts, should be studied in detail.

In contrast to the L929 fibroblasts, mitochondrial activity of the THP1 monocytes was uniformly unaffected by the metal-APT materials (Fig. 4). This resistance was surprising considering that each of the metals or metal compounds alone suppressed mitochondrial activity (Fig. 2), albeit sometimes at a higher concentration than for fibroblasts. These results suggest that cell-APT interactions may be cell-type dependent, and that therapeutically, the use of metal-APT materials may be somewhat selective to certain cell types in a tissue. Alternatively, because the APT complexes are denser than water, the higher susceptibility of the anchorage-dependent fibroblasts to the metal-APT particles vs. the monocyte suspensions may

have resulted from a relatively inefficient interaction of APT particles with the suspended monocytes vs. the fixed fibroblasts at the bottom of the culture wells. Another less likely possibility was that the different cell-culture media caused different extracellular release rates of metal, accounting for the lack of THP1 response. In any case, the lack of 'toxicity' of the metal-APT materials toward THP1 provides an opportunity to assess the effects of the materials on other monocytic functions. Overall, the differential response of cell types to the metal-APT complexes suggests that certain types of cells might be targeted or avoided during APT-mediated local delivery of metal based drugs.

5. Conclusions

Our data suggest that metal-loaded amorphous peroxotitinates (APT) deliver metals to affect cellular function of some, but not all types of cells. This 'biodelivery' likely involves a complex interaction between the cell and the metal-APT, rather than simply from extracellular release of the metal compound. Our results here support further study into the use of metal-APT complexes as novel delivery vehicles for metal-based ions or compounds that could be sequestered by biological or synthetic barriers to reduce the systemic toxicity experienced with current metal-based therapeutics. The current work provides support for investigation into how these complexes alter cell behavior (e.g., via extracellular vs. intracellular release), how different tissues may react, and identification of the most promising metal-based agents for affecting changes in a variety of cellular functions.

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Figure Legends

Fig. 1. Amorphous peroxotitanate (APT). *Upper left:* powder; *lower left:* 15 wt% aqueous suspension; *upper right:* SEM image, 400x magnification of powder; *lower right:* SEM image, 5030x magnification of powder.

Fig. 2. Dose-response curves of THP1 monocytes or L929 fibroblasts to metal ions and compounds alone (Table 1). Cellular succinate dehydrogenase (SDH) activity was measured and expressed as a percentage of controls receiving no metal. The dash horizontal line indicates control levels of SDH activity, arbitrarily set to 100%. Error bars represent standard deviations of replicates (n = 8).

Fig. 3. Dose-response curves of L929 fibroblasts to Hg(II), CisPt, and Pt(IV) alone (Table 1). The results from Fig. 2 were repeated for these metals with a lower concentration range. Cellular succinate dehydrogenase (SDH) activity was measured and expressed as a percentage of controls receiving no metal. The dash horizontal line indicates control levels of SDH activity, arbitrarily set to 100%. Error bars represent standard deviations of replicates (n = 8).

Fig. 4. THP1 SDH activity in response to added amorphous peroxotitanate (APT)-metal complexes (loaded APT, see Table 2) vs. APT alone (red) after 72 h exposure. Error bars indicate standard deviations of n = 8. There were no statistically significant differences between the controls (horizontal dashed line, red line) and addition of the metal complexes (ANOVA, α = 0.05).

Fig. 5. L929 fibroblast SDH activity in response to amorphous peroxotitanate (APT)-metal complexes (loaded APT, see Table 2) vs. APT alone (red) after 72 h exposure. Error bars indicate standard deviations of n = 8. Lower-case letters delineate statistical groupings (ANOVA, Tukey post-hoc, α = 0.05). Horizontal line indicates native cell SDH activity.

Fig. 6. Possible mechanism(s) of biodelivery of metal (M) compounds from metal-APT materials

to cells. The mechanism (top) involving pure extracellular release of the metal compound is not supported by the data in the current paper (Table 3, Figs. 2 and 5).

Table 1: Metal Compounds, Sources, Metal Loading Concentrations

Metal Species	Source compound	Manufacturer	Metal loading solution conc. (μM)
Gd(III)	$Gd(NO_3)_2 \cdot 6H_2O$	Aldrich Chemical Company, Inc. (St. Louis, Mo)	11,200
Hg(II)	$Hg(NO_3)_2 \cdot H_2O$	Fisher Scientific (Pittsburgh, PA)	18,900
Pd(II)	$PdCl_2$	Johnson Matthey, Inc. (West Chester, PA)	27,400
Pt(IV)	$PtCl_4$	Johnson Matthey, Inc. (West Chester, PA)	15,100
cisPt	cis-$[PtCl_2(NH_3)_2]$	Alfa-Aesar (Ward Hill, MA)	6,100

Table 2: Degree of metal-APT (amorphous peroxotitanate) loading

Metal Ion	Metal loading solution conc.* (μM)	APT loading ** (fmol/μg-APT)
Gd(III)	11,200****	78.8 (8)***
Hg(II)	18,900	37.8 (4)
Pd(II)	27,400	142 (15)
Pt(IV)	15,100	18.0 (2)
cisPt	6,100	56.8 (6)

* initial metal concentration used to prepare metal-APT materials
** based on solution analyses before and after contact with APT
*** uncertainties calculated using propagation of error techniques
**** concentration in dilute nitric acid solution (pH 3)

Table 3: Comparison of expected SDH suppression by APT* vs. actual SDH suppression.

Metal Species	Max. metal delivery from metal-APT** (μM)**	$TC50_{SDH}$*** L929 (μM)	Actual metal-APT induced SDH Suppression**** (%)
Gd(III)	7.9	> 500	45
Hg(II)	3.8	18 (4)	40
Pd(II)	14.2	100 (20)	30
Pt(IV)	1.8	28 (3)	50
cisPt	5.7	< 1	65

* APT = amorphous peroxotitanate
** based on 100% delivery of 100 μg/mL of loaded APT into 200 μL cell-culture medium.
*** Concentration in μM required to suppress SDH activity by 50%, based on dose-response curve with metals alone, determined graphically from data in Figs 2,3.
**** Maximum suppression of SDH activity by metal-APT materials from Fig. 5 (L929)

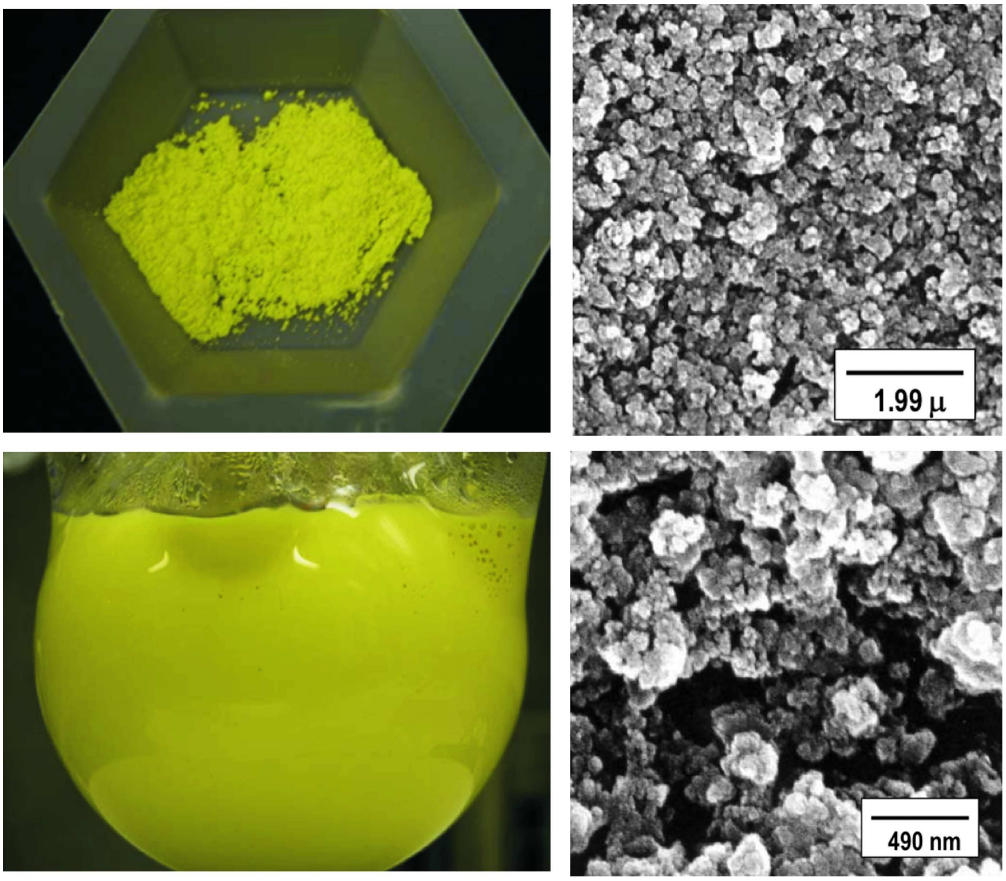

Fig. 1. Amorphous peroxotitanate (APT). Upper left: powder; lower left: 15 wt% aqueous suspension; upper right: SEM image, 400x magnification of powder; lower right: SEM image, 5030x magnification of powder.
120x105mm (600 x 600 DPI)

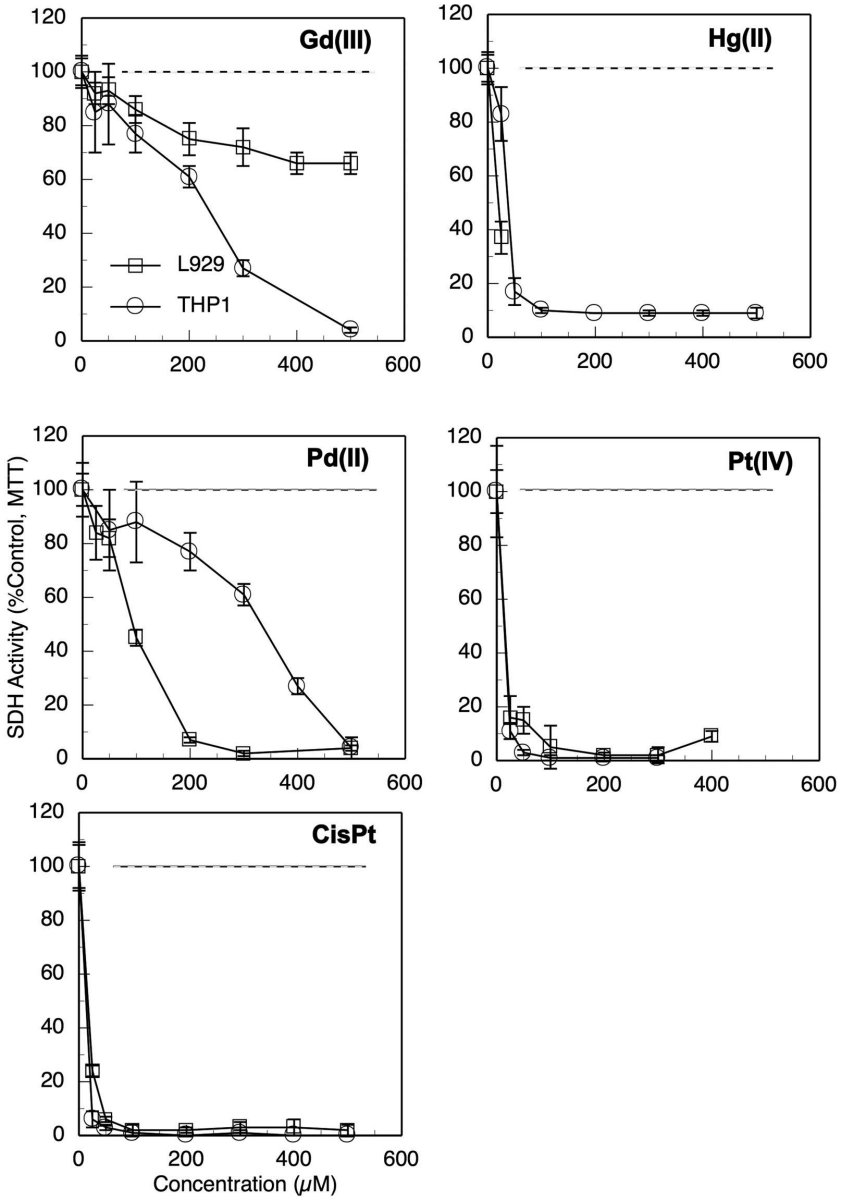

Fig. 2. Dose-response curves of THP1 monocytes or L929 fibroblasts to metal ions and compounds alone (Table 1). Cellular succinate dehydrogenase (SDH) activity was measured and expressed as a percentage of controls receiving no metal. The dash horizontal line indicates control levels of SDH activity, arbitrarily set to 100%. Error bars represent standard deviations of replicates (n = 8).
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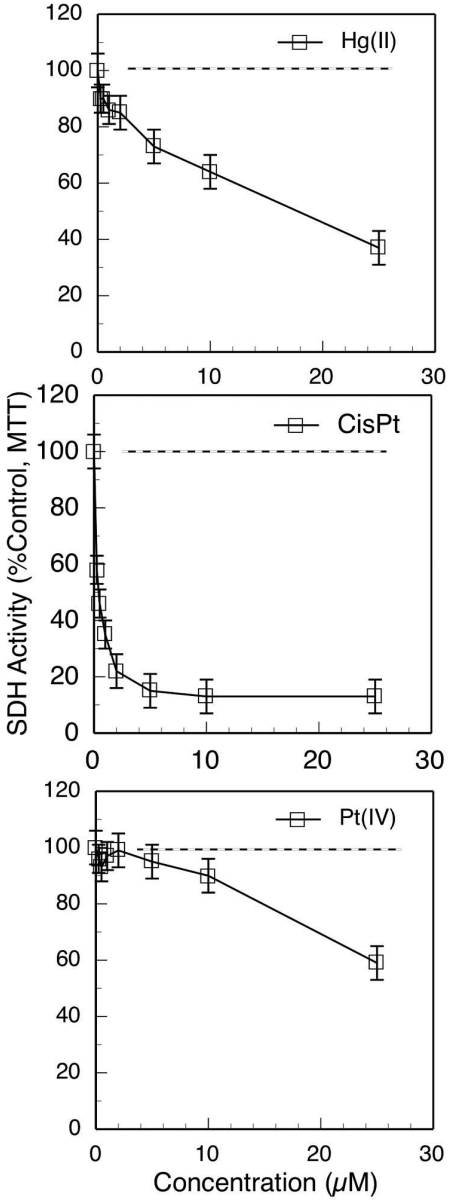

Fig. 3. Dose-response curves of L929 fibroblasts to Hg(II), CisPt, and Pt(IV) alone (Table 1). The results from Fig. 2 were repeated for these metals with a lower concentration range. Cellular succinate dehydrogenase (SDH) activity was measured and expressed as a percentage of controls receiving no metal. The dash horizontal line indicates control levels of SDH activity, arbitrarily set to 100%. Error bars represent standard deviations of replicates (n = 8).
63x170mm (1200 x 1200 DPI)

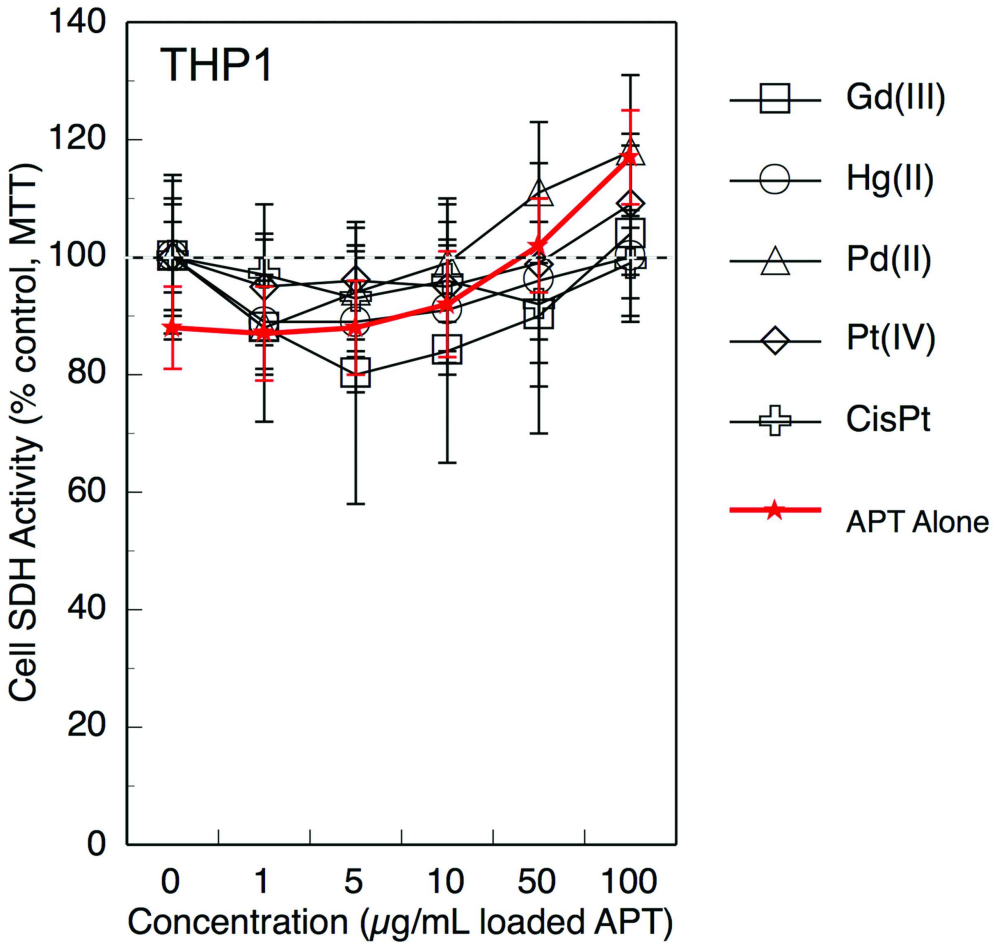

Fig. 4. THP1 SDH activity in response to added amorphous peroxotitanate (APT)-metal complexes (loaded APT, see Table 2) vs. APT alone (red) after 72 h exposure. Error bars indicate standard deviations of n = 8. There were no statistically significant differences between the controls (horizontal dashed line, red line) and addition of the metal complexes (ANOVA, α = 0.05).
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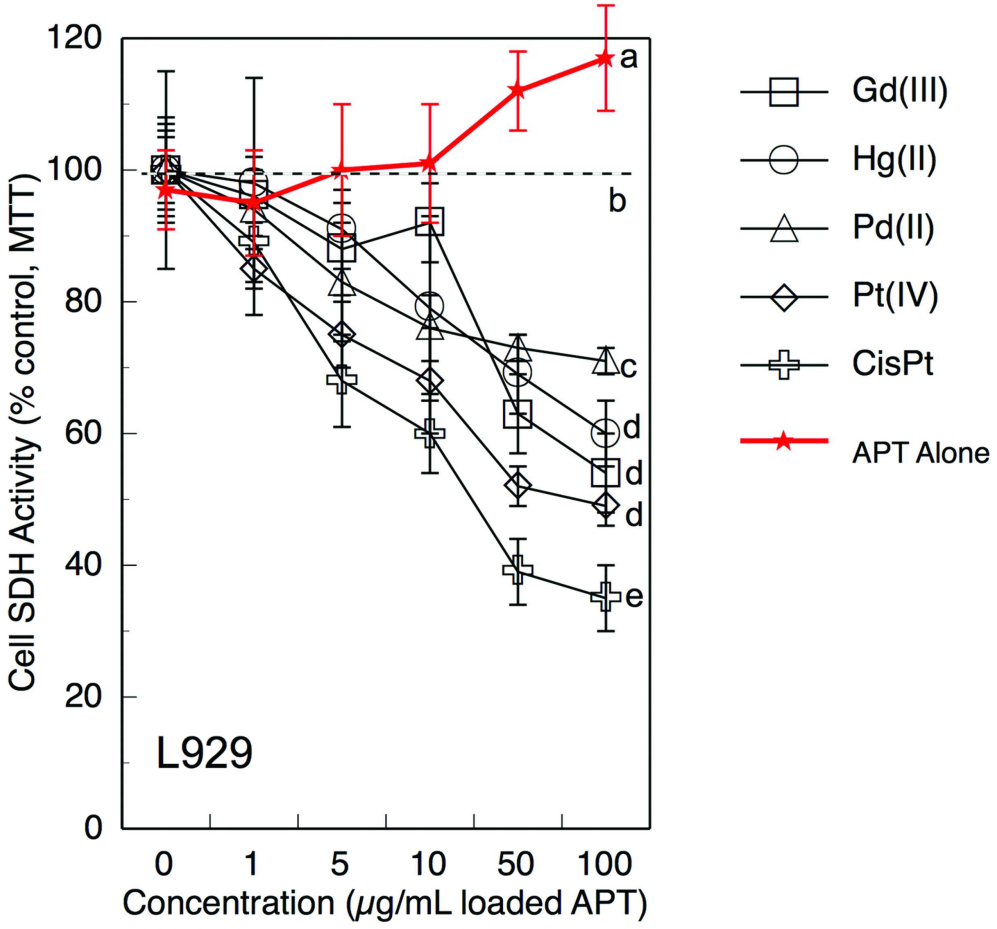

Fig. 5. L929 fibroblast SDH activity in response to amorphous peroxotitanate (APT)-metal complexes (loaded APT, see Table 2) vs. APT alone (red) after 72 h exposure. Error bars indicate standard deviations of n = 8. Lower-case letters delineate statistical groupings (ANOVA, Tukey post-hoc, α = 0.05). Horizontal line indicates native cell SDH activity.
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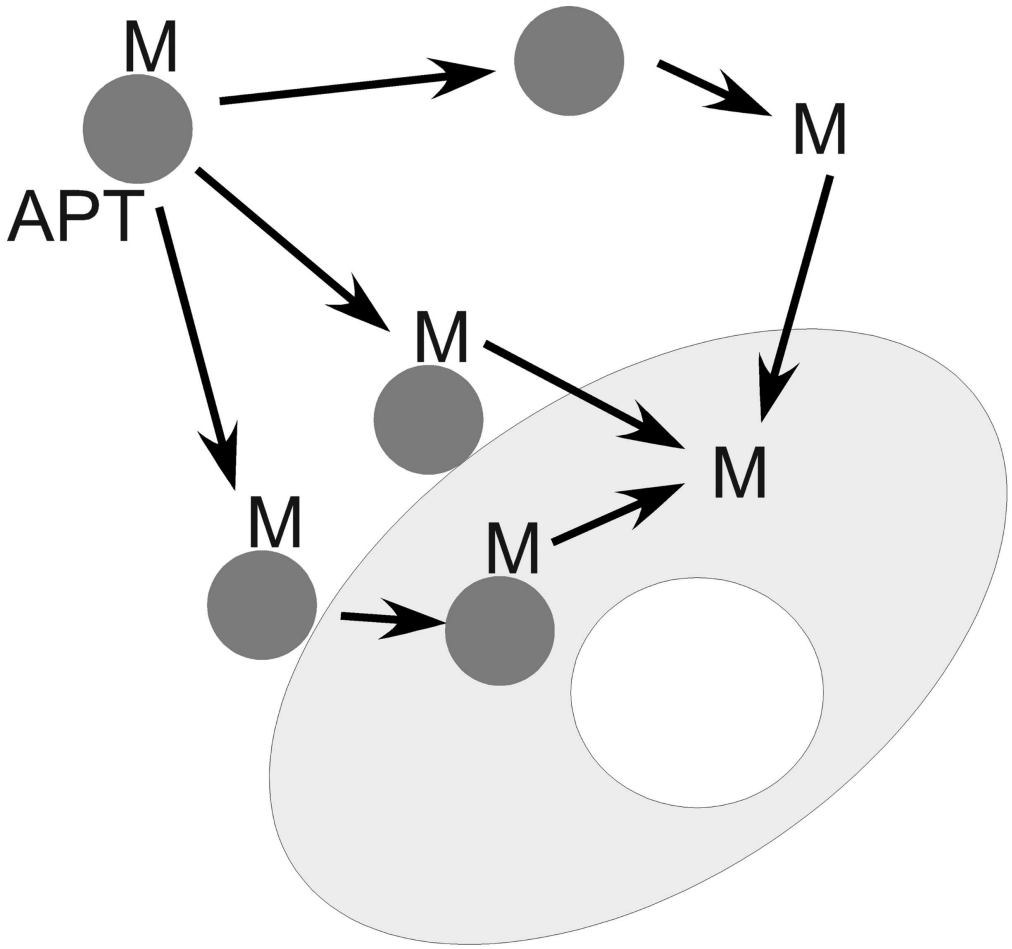

Fig. 6. Possible mechanism(s) of biodelivery of metal (M) compounds from metal-APT materials to cells. The mechanism (top) involving pure extracellular release of the metal compound is not supported by the data in the current paper (Table 3, Figs. 2 and 5).
102x96mm (600 x 600 DPI)