J Exp Clin Med 2014;6(1):21–27

Contents lists available at ScienceDirect

Journal of Experimental and Clinical Medicine

journal homepage: http://www.jecm-online.com

ORIGINAL ARTICLE

Titanates Deliver Metal Compounds to Suppress Cell Metabolism

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ARTICLE INFO

Article history:
Received: Sep 7, 2013
Accepted: Apr 1, 2014

KEY WORDS:
mitochondrial activity;
OSC2 cells;
titanates

Background: Titanates are inorganic, amorphous, aqueously insoluble, particulate compounds of titanium, oxygen, hydrogen, and sodium. Two types of titanates, monosodium titanate (MST) and amorphous peroxotitanate (APT), have recently been proposed for use in biological applications where it would be advantageous to locally sequester and deliver metals to alter cellular functions.
Purpose: In the current study, the ability of MST and APT to suppress the mitochondrial function of a rapidly dividing cell line, OSC2 (oral squamous cell carcinoma) with and without loaded metals [Au(III), Hg(II), Pd(II), Pt(II), Pt(IV), and *cis*Pt] was determined.
Methods: Cellular mitochondrial activity of OSC2 cells after 72 hours exposure to titanates or titanate-metal compounds was estimated by measuring succinate dehydrogenase (SDH) activity via the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.
Results: The results of this study showed that neither native APT nor native MST was biologically neutral to oral squamous cell carcinoma (OSC2). Increasing the concentration of either MST or APT resulted in a statistically significant decrease in SDH activity of OSC2 cells versus untreated cells (40% for APT and 30% for MST). The addition of titanate-metal compounds augmented the effects of APT and MST on OSC2 metabolism, decreasing overall mitochondrial activity compared to controls by 50–70%. However, both titanate types and metal ion species determined the degree to which the SDH activity was suppressed.
Conclusion: The findings of the current study demonstrate that APT and MST alone significantly suppress the metabolism of a rapidly dividing cell line, an effect that is augmented by specific titanate-metal combinations. These compounds may have potential as unique therapeutic agents.

1. Introduction

Metals have appeal for novel drugs because of their unique binding and redox properties.[1] Currently, the two most therapeutically employed metals are Au(I) in the form of Auranofin for the treatment of arthritis[2] and platinum-based [Pt(II)] drugs for the treatment of several cancers.[3–5] Predominantly, the systemic toxicity of metals has limited their applicability; one possible solution to this limitation is to localize the delivery of therapeutic metals. To this end, two titanate materials, monosodium titanate (MST) and amorphous peroxotitanate (APT), have been explored for use in biological environments where it would be advantageous to locally sequester and deliver metals for therapy.[6–10]

MST is an inorganic, amorphous, aqueously insoluble, particulate compound originally developed for the decontamination of nuclear waste. In this application, dissolved radioactive species are adsorbed onto MST, then separated from the aqueous phase by centrifugation.[11] APT is a closely related material that exhibits faster sorption kinetics.[12] MST and APT have spherical morphologies with an approximate diameter of 2–20 μm. Whereas initially synthesized to bind strontium and actinides in nuclear waste, these particles also bind biologically important metal ions and metal complexes, such as gold, Auranofin, mercury, palladium, gadolinium, platinum, and cisplatin.[7,13]

In studies to date, both APT and MST in their native forms are relatively innocuous to several mammalian cell types. For example, neither APT nor MST significantly increase or reduce the mitochondrial [succinate dehydrogenase (SDH)] activity of murine L929 fibroblasts or human THP1 monocytes.[6–8] Yet metal-loaded forms cause metal-specific and cell-specific changes in cell metabolism. For example, treatment of L929 fibroblasts with titanate-gold compounds decreased cellular mitochondrial metabolism 30–80% in a dose-dependent fashion.[7,8] With the exception of cisplatin,

Conflicts of interest: All authors declare no conflicts of interest.

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1878-3317/$ – see front matter
http://dx.doi.org/10.1016/j.jecm.2014.01.010

these decreases occur at metal ion concentrations much lower than those required to achieve decreases with the corresponding metals alone, suggesting that the titanates facilitate metal delivery to the cells.

Overall, the studies completed thus far demonstrate a potential for further development of MST and APT as vehicles for localized delivery of metal ions in biological environments. Accordingly, the current study tested a hypothesis that APT and MST deliver metal ions to suppress the metabolism of rapidly dividing cells, extending previous work with more slowly dividing cell types. Here, the ability of MST and APT to suppress the mitochondrial functions of an oral cancer cell line, OSC2 (oral squamous cell carcinoma), with and without loaded metals [Au(III), Hg(II), Pd(II), Pt(II), Pt(IV), and *cis*Pt] was measured.

2. Materials and methods

2.1. Titanates and titanate-metal loading

The current investigation focused on Au(III), Hg(II), Pt(IV), Pt(II), cisplatin, and Pd(II), because these metal ions have been used or proposed for therapeutic roles in medicine. MST and APT with or without gold [Au(III)], palladium [Pd(II)], mercury [Hg(II)], platinum [Pt(II) and Pt(IV)], and cisplatin (*cis*Pt) (Table 1) were assessed for their ability to suppress the growth of rapidly dividing cells. MST was obtained commercially (Optima Chemical Group, LLC Douglas, GA, USA) and APT was synthesized from MST as previously described.[6,12]

Metal-loaded forms of APT and MST were synthesized to maximize metal loading without attempting to equalize the amount of metal adsorbed to the titanate. Loaded titanates were prepared by combining 0.25 g APT or MST suspended in 1.4 g water (pH = 6.9) with 10 mL of phosphate buffered saline solution containing the metal ion or metal complex (Table 1) for 48 hours at room temperature. The titanate-metal solid was then separated from the solution via centrifugation (rpm = 1200*g* for 3 minutes). The resulting solid phase was rinsed six times with chilled phosphate buffered saline (4°C, pH = 7.4) and centrifuged (1200*g* for 3 minutes). The clear supernatant liquid was then removed by transfer pipette and the moist solid was stored with approximately 75 wt% water content.

The quantity of metal loaded onto the titanates was determined by measuring the difference in metal concentrations between the loading solution before and after contact with the titanate. Inductively coupled plasma mass spectrometry was used to determine metal ion concentration in each solution except mercury, for which cold vapor atomic adsorption spectroscopy was used. The amount of loaded metal was reported as g of metal/g of titanate (Table 1).

2.2. Cells and cell-culture

A human oral squamous cell carcinoma cell line (OSC2, gingival squamous cell carcinoma from Dr. Tokio Osaki, Kochi Medical School, Kochi, Japan) was used in this study to test the ability of titanate-metal complexes to suppress the mitochondrial activity of rapidly dividing cells. The OSC2 cells were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium:F12, supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 5 μg/mL hydrocortisone (Sigma-Aldrich, St. Louis, MO, USA; all other reagents from Invitrogen-Life Technologies, Grand Island, NY, USA). Cells were maintained in an incubator at 37 °C, 5% CO_2, and 100% relative humidity.

Cells were plated in 96-well format (n = 8, flat-bottom) at a density of 5000 cells/cm^2 in 200 μL of culture media. The plated cells were incubated for 24 hours to allow for adherence prior to exposure to metals, APT, MST, APT-metal, or MST-metal suspensions. The cytotoxicity of a range of metal ion dilutions (0–300 μM) was assessed by adding a 10 μL volume of PBS-metal ion solution to each culture well. The metal dilution range was dependent on several factors, including metal solubility and toxicity to the cells. Following the addition of metal ions, cells were incubated for 72 hours, at which point, the mitochondrial activity of the treated cells was measured via SDH activity (see next section: *Cell response*).

Similarly, titanates and metal-titanate complexes were diluted from stock suspensions to concentrations that resulted in 0 μg/mL, 0.5 μg/mL, 1 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL final concentrations when 10 μL was added to 200 μL media in each well. Again, the treated cultures were incubated for 72 hours prior to measuring SDH.

2.3. Cell response

Cellular mitochondrial activity was assessed by measuring SDH activity via the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[14] Briefly, 100 μL of 2% MTT solution, 0.05 M Tris, 0.5 mM $MgCl_2$, 2.5 mM $CoCl_2$, 0.25M disodium succinate (all reagents from Sigma-Aldrich) were added to each well. The cells were then incubated for 45 minutes at 37°C. Following incubation with the MTT solution, 100 μL of 4% formalin (Sigma-Aldrich) in 0.2 M Tris were added to each well and allowed to react for 3–5 minutes. Upon removal of the MTT-Tris-formalin solution, each well was rinsed with 200 μL/well water (18 MOhm) and air dried for 5 minutes. Finally, the MTT-formazan product was solubilized with 100 μL/well of 6.25% (v/v) dimethylsulfoxide (Fisher Chemical, Pittsburg, PA, USA) in 0.1 N NaOH (Fisher Chemical) and mixed to homogeneity prior to reading the optical density at a wavelength 562 nm using a Spectramax M2 plate reader (Molecular Devices, Sunnyvale, CA, USA). Each experiment was completed in triplicate. The statistical significance of metal- or titanate-induced effects was determined using one-way analysis of variance and Tukey pairwise multiple comparison intervals, with $\alpha = 0.05$.

2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was used, on a pilot basis, to begin to investigate any interactions between the titanate particles and the OSC2 cells. Silicon segments with an approximate dimension of 1 cm × 1 cm were submerged in cell-culture medium in 8-

Table 1 Metal compounds, sources, loading concentrations, and amorphous peroxotitanate (APT)- and monosodium titanate (MST)-loaded concentrations

Metal species	Source compound	Manufacturer	Titanate loading concentration (μM)	Loaded concentration (g metal/g APT)	Loaded concentration (g metal/ g MST)
Au(III)	$HAuCl_3.3H_2O$; MW = 393.83	Sigma-Aldrich (St. Louis, MO, USA)	13,300	0.0852	0.0789
Hg(II)	$Hg(NO_3)_2.H_2O$; MW = 342.62	Sigma-Aldrich (St. Louis, MO, USA)	18,900	0.0499	0.120
Pd(II)	$PdCl_2$; MW = 177.33	Johnson Matthey, Inc. (West Chester, PA, USA)	13,200	0.0539	0.0557
Pt(II)	$PtCl_2$; MW = 265.98	Johnson Matthey, Inc. (West Chester, PA, USA)	114	0.00086	0.00084
*cis*Pt	cis-$[PtCl_2(NH_3)_2]$; MW = 790.56	Alfa-Aesar (Ward Hill, MA, USA)	4460	0.0284	0.0341
Pt(IV)	$PtCl_4$; MW = 336.89	Johnson Matthey, Inc. (West Chester, PA, USA)	14,900	0.0155	0.0686