Tetrachloroaurate (III)–induced Oxidation Increases Non-thermal Plasma-induced Oxidative Stress

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Abstract

Non-thermal plasma (NTP) devices have been explored for medical applications. NTP devices discharge electrons, positive ions, ultraviolet, and reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as the hydroxyl radical ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$), superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), ozone, and nitric oxide, at near-physiological temperature. At preclinical stages or in human clinical trials, NTP promotes blood coagulation, eradication of bacterial, viral, and biofilm-related infections, wound healing, and cancer cell death. Here, we observed that ferric, vanadium, and gold(III) ions, measured by 2-thiobarbituric acid-reactive substances (TBARS) in combination with NTP exposure, significantly elevated lipid peroxidation. Using 3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide (M4PO) as a spin probe in electron paramagnetic resonance (EPR), we observed that tetrachloroaurate (III) yielded an M4PO-X spin adduct. Tetrachloroaurate-induced oxidation was attenuated efficiently by reduced (GSH) and oxidized glutathione (GSSG), while glycine (Gly) and *L*-glutamate (Glu), components of GSH, were ineffective. Furthermore, GSH and GSSG efficiently suppressed tetrachloroaurate-induced lipid peroxidation, while Gly and Glu were ineffective in suppressing TBARS elevation. These results indicate that tetrachloroaurate-induced oxidation is attenuated by GSH as well as GSSG. Further studies are warranted to elucidate the redox reactions between metal ions and biomolecules to advance the clinical application of NTP.

Introduction

The medical application of non-thermal plasma (NTP) originated in the late 1970s and has been improving since the mid-1990s¹. NTP is composed of electrons, various ions, ultraviolet (UV), and reactive oxygen/nitrogen species (ROS/RNS), such as ozone (O_3), hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), superoxide ($\text{O}_2^{\bullet-}$), the hydroxyl radical ($\bullet\text{OH}$), and nitric oxide (NO). These molecules initiate blood coagulation, eradicate bacterial, viral, and biofilm-related infections and promote inorganic surface optimization, wound healing, and cancer cell death²⁻⁴. Recently, CE-certified NTP devices have been available for disinfection (bacteria/fungi/viruses), atopic eczema, and wound healing. Furthermore, clinical studies on advanced head and neck squamous cell carcinomas and malignant melanoma have been conducted⁵. However, the biological outcomes of NTP may be affected by the generated levels of ROS/RNS, which depend on the design and operation of NTP sources. Thus, there is a need to optimize conditions to achieve the ideal biological effects.

To overcome the limitation of direct NTP exposure, NTP-activated medium (PAM)⁶⁻¹⁰ or NTP-activated Ringer's lactate (PAL)¹¹⁻¹⁵ have been explored for eradicating cancer cells, and results demonstrated that PAM and PAL effectively kill a wide range of tumors. However, the biological role of ROS/RNS in selective killing is yet to be elucidated. Previously, we have demonstrated that NTP simultaneously generates H_2O_2 , $^1\text{O}_2$, $\text{O}_2^{\bullet-}$, $\bullet\text{OH}$, and UV modifications¹⁶⁻¹⁹. Using this device, selective killing was demonstrated in oral squamous cell carcinoma²⁰, glioblastoma²¹, ovarian adenocarcinoma²², and malignant

mesothelioma^{23,24}. Indeed, iron, manganese, copper, lead, cobalt, mercury, beryllium, nickel, cadmium, molybdenum, tin, titanium, vanadium, chromium, aluminum, selenium, and arsenic have been shown to increase oxidative injury²⁵. However, the intensity at which NTP-induced oxidative injury is amplified remains to be elucidated. In this study, we screened soluble element ions, which could enhance lipid peroxidation determined using a 2-thiobarbituric acid-reactive substances (TBARS) assay. Using this screening, we determined that ferric, vanadium, and gold (III) ions elevated TBARS, and electron paramagnetic resonance (EPR) showed that tetrachloroaurate (HAuCl_4) yielded an oxidized spin adduct. These results reveal the utility of metal ions in medical NTP application.

Results

Determination of PC-derived TBARS after NTP exposure for 2 min in the presence of ions

In the screening system, we observed that NTP irradiation significantly elevated TBARS and identified ferric, vanadium, and gold (III) ions as candidates that amplify NTP-induced oxidative stress (Fig. 1A). In the first step of screening, many samples were irradiated simultaneously, causing auto-oxidation in the air. The tendency of TBARS to increase in Ar gas-treated samples than in untreated samples may be due to spraying in the air. To clarify the overload of NTP-induced ROS in metal-mediated auto-oxidation, a limited number of experimental samples was measured simultaneously. In these experiments, NTP significantly elevated TBARS in ferric (Fig. 1B), vanadium (Fig. 1C), and gold (III) ions (Fig. 1D) relative to Ar gas treatment. Notably, the metal ions elevated TBARS in a dose-dependent manner (Fig. 1B–D), indicating that ambient air-induced oxidation is an important factor in the present experimental setting.

Tetrachloroaurate (HAuCl_4) yields an oxidized spin adduct (M4PO-X)

Exposure to NTP for 2 min without tetrachloroaurate generated M4PO-OH and M4PO-H spin adducts (Fig. 2A), as previously observed^{17,19}. With an increase in tetrachloroaurate, NTP-induced M4PO-OH decreased (Fig. 2A). This tendency was also observed in DMPO-H and DMPO-OH in the presence of tetrachloroaurate (**supplementary Fig. 1**). On the other hand, a triplet signal was observed from 5 μM tetrachloroaurate in the Ar gas treatment (Fig. 2B). M4PO-X production was elevated in accordance with the concentration of tetrachloroaurate in the NTP and Ar gas treatments (Fig. 2B). The formation of M4PO-X was significantly higher in Ar gas than in NTP, indicating that NTP decayed M4PO-X as observed in other spin probes¹⁸. DMPO-X was not detected (**Supplementary Fig. 1**).

GSH and GSSG significantly attenuate tetrachloroaurate (HAuCl_4)-induced oxidation

GSH partially attenuated the tetrachloroaurate-induced oxidized spin adduct at 25 μM and completely at 250 μM in the Ar gas treatment (Fig. 3A). GSH (100 μM) with NTP irradiation decayed M4PO-X and

M4PO-OH simultaneously, while 250 μ M GSH with NTP irradiation generated M4PO-OH only (Fig. 3A) as is the case without tetrachloroaurate¹⁷. Gly and Glu, which are constituents of GSH, did not attenuate tetrachloroaurate-induced oxidation effectively (Fig. 3B, C). On the other hand, GSSG attenuated tetrachloroaurate-induced oxidation significantly from 10 μ M (Fig. 3D). As observed in GSH, 100 and 250 μ M GSSG with NTP generated M4PO-OH (Fig. 3D).

GSH and GSSG significantly attenuate tetrachloroaurate (HAuCl₄)-induced lipid peroxidation

In the Ar gas and NTP treatments, 100 and 250 μ M GSH significantly suppressed lipid peroxidation in the presence of 100 μ M tetrachloroaurate (Fig. 4A, B). GSH (25, 50, 100, and 250 μ M) significantly suppressed lipid peroxidation in the presence of 50 μ M tetrachloroaurate. GSH (10, 25, 50, 100, and 250 μ M) significantly suppressed lipid peroxidation in the presence of 10 μ M tetrachloroaurate, while there was no significant lipid peroxidation suppression without tetrachloroaurate (Fig. 4A, B). In the Ar gas and NTP treatments, Gly was ineffective in suppressing lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate and in its absence (Fig. 4C, D). Similar observations were made with Glu under the same conditions (Fig. 4E, F). In the Ar gas treatment, 100 and 250 μ M GSSG significantly suppressed lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate (Fig. 4G), whereas in the NTP treatment, 250 μ M GSSG significantly suppressed lipid peroxidation under similar conditions. Furthermore, 100 μ M GSSG significantly suppressed lipid peroxidation in the presence of 10 μ M tetrachloroaurate (Fig. 4H). There was no significant suppression of lipid peroxidation without tetrachloroaurate in all of the examined compounds in the present study (Fig. 4), consistent with the EPR results (**Supplementary Fig. 2**) and previous reports^{17,18}.

Discussion

Here, we determined that tetrachloroaurate oxidizes M4PO and liposome by screening for metal ions that amplify NTP-induced lipid peroxidation. As a result, we observed that ferric and vanadium ions induced higher lipid peroxidation than tetrachloroaurate (Fig. 1B–D). Ferric ions cause oxidative injury to cells that ultimately causes carcinogenesis in mammals²⁶. Contrastingly, in plasma medicine, iron is used for the therapeutic purpose of killing cancer cells^{6,12,20,23,24}. Vanadium exists in several oxidation states, including V(V), V(IV), V(III), and V(II), among which V(IV) is a major oxidation state in crude oil and clay rock²⁷. Regarding therapeutic application, the insulin-like effects that maintain signal transduction for glucose uptake and enhance lipid metabolism have been investigated extensively²⁷. In addition, vanadium is known to catalyze the generation of superoxide, $[V(IV) + O_2 \rightleftharpoons O_2^{\bullet-} + V(V)]$ and the hydroxyl radical from H₂O₂ $[V(IV) + H_2O_2 \rightarrow V(V) + OH^- + \bullet OH]$ ^{25,28,29}. Further studies on other vanadium salts are needed to explore the utility of vanadium with NTP application. Other transition elements have been reported to catalyze the ‘Fenton reaction’ to generate $\bullet OH$ from H₂O₂; however, these did not dramatically increase lipid peroxidation in our screening.

Tetrachloroaurate-induced oxidation yielded M4PO-X, which is characterized by a triplet signal³⁰. This M4PO-X spin adduct reached a plateau at pH 4.0–6.0 and decreased to 40% at pH 7.0, finally becoming undetectable at pH 9.0. DMPO-X, which reacted 7-fold faster than M4PO-X, was also yielded by tetrachloroaurate at pH 4.0, while relative intensity of the EPR signal for DMPO-X was 30–40 % lower than that for M4PO-X at pH 4.0–7.0³⁰. Here, we did not detect DMPO-X with 100 μ M tetrachloroaurate after Ar gas treatment for 2 min at pH 7.4 (**Supplementary Fig. 1**). Meanwhile, the characteristic signals of M4PO-X and DMPO-X spin adducts were observed following reaction with chlorine dioxide ($\text{ClO}_2^\bullet$) at pH 2.0 and that of Ti^{3+} with potassium chlorate (KClO_3)³¹. These results suggest that tetrachloroaurate-induced oxidation may be associated with $\text{ClO}_2^\bullet$, especially under acidic conditions. Here, tetrachloroaurate-induced M4PO-X was generated in a dose-dependent manner, decaying after NTP exposure (Fig. 2B). On the other hand, in the presence of amino acids and dipeptide, such as Gly (Fig. 3B), Glu (Fig. 3C), and *L*-alanine (Ala) with *L*-glutamine (Gln) (**Supplementary Fig. 3A**), NTP irradiation did not significantly decay the M4PO-X spin adduct. These results suggest that amino acids may stabilize the M4PO-X spin adduct. The rate constant for the reaction between Au(III) and Gly and Ala was less than $100 \text{ M}^{-1} \text{ s}^{-1}$ ^{32,33}. Furthermore, Au(III) reacts with two GSH to form reduced Au(I) and GSSG or GSH sulfonic acid that may disrupt normal biological function by altering the secondary and tertiary structures of a protein^{34,35}. On the other hand, gold nanoparticles generated $^\bullet\text{OH}$ under microwave irradiation³⁶ so did gold nanocages in photodynamic therapy³⁷, indicating that ionized gold status is critical for gold-catalyzed oxidation.

Tetrachloroaurate significantly induced lipid peroxidation (Fig. 4). The NTP irradiation overloaded ROS in tetrachloroaurate-induced oxidation (Fig. 4B, 4D, 4F, 4H). The supplementation of GSH and GSSG significantly suppressed tetrachloroaurate-induced elevation of TBARS in a dose-dependent manner (Fig. 4A, 4B, 4G, 4H). In contrast, the TBARS were not suppressed by GSH or GSSG at up to 250 μ M after NTP irradiation without tetrachloroaurate, suggesting that $^\bullet\text{OH}$ is not scavenged effectively by these compounds. Taken together, the reactivity of tetrachloroaurate is presumed to be the order of PC, GSH > GSSG > M4PO. Meanwhile, Gly and Glu, which are constituents of GSH, as well as Ala-Gln, did not suppress NTP-induced lipid peroxidation (Fig. 4C–F **and Supplementary Fig. 3C, D**). Furthermore, Gly, Glu, and Ala-Gln were not significantly scavenged $^\bullet\text{OH}$ (**Supplementary Fig. 2 and Supplementary Fig. 3B**). These TBARS assay results are consistent with those of EPR (Fig. 3), indicating that thiol and disulfide bonds attenuated tetrachloroaurate-induced oxidation. Recently, we observed that GSSG reacted with NTP-induced H_2O_2 ¹⁸. Further study may reveal the role of disulfide bonds in ROS scavenging.

Gold exists in several oxidation states from I to V; among these, only Au(I/III) have been used for medication³⁸. Triethylphosphine gold (I) chloride, an analog of auranofin, induced electron-dense deposition within mitochondria, lipid peroxidation, and depletion of GSH and NADPH in rat primary hepatocytes³⁹. Recently, Au(I/III) complexes inhibited thioredoxin reductases (TrxRs) with high potency and specificity through covalent interaction with the selenocysteine-containing active site and potentially reacted with Cys residues of GSH reductase (GR), GSH peroxidases (GPxs)^{38,40}, and *L*-histidine residues

of ferritin heavy chain³². Furthermore, depending on the chelating structure, Au(III) complexes inhibited growth in invading microbes⁴¹ and cellular proliferation in cancer cells⁴². These results indicate that Au(I/III) trigger metal-induced oxidative stress and simultaneously disrupt the antioxidative defense system (TrxRs, GR, and GPxs). Moreover, auranofin initiated ferroptosis, which is induced by GPx4 inactivation and suppressed by iron chelating, in *MYCN*-amplified neuroblastoma⁴³, suggesting the presence of an interaction between iron and gold-induced oxidative stress.

In conclusion, we observed that NTP-induced ROS and tetrachloroaurate-induced oxidation synergistically increase lipid peroxidation, which is attenuated by GSH and GSSG. Thus, combining NTP with metal ions, based on an understanding of redox mechanisms, could expand the possibility of therapeutic applications.

Materials And Methods

Chemicals. The TBARS assay kit was purchased from Oxford Biomedical Research (Oxford, MI, USA). Phosphatidylcholine (PC) from egg yolk, GSH, GSSG, *L*-glutamate (Glu), glycine (Gly), standard solutions for atomic absorption spectrochemical analysis; sodium (NaCl), potassium (KCl), germanium (GeO₂), silicon (Na₂SiO₃), magnesium [Mg(NO₃)₂], calcium (CaCO₃), strontium (SrCO₃), barium (BaCO₃), manganese [Mn(NO₃)₂], chromium (K₂Cr₂O₇), iron [Fe(NO₃)₃], cobalt [Co(NO₃)₂], nickel [Ni(NO₃)₂], copper [Cu(NO₃)₂], zinc [Zn(NO₃)₂], silver (AgNO₃), cadmium [Cd(NO₃)₂], lead [Pb(NO₃)₂], aluminum [Al(NO₃)₃], gallium (Ga) in 0.5 M HNO₃, vanadium (V) in 0.2 M HCl and 0.5 M HNO₃, molybdenum (Mo) in 0.4 M HCl and 0.2 M HNO₃, scandium [Sc(NO₃)₃], neodymium [Nd(NO₃)₃], gadolinium [Gd(NO₃)₃], erbium [Er(NO₃)₃], platinum (H₂PtCl₆), gold (HAuCl₄), and titanium [Ti(SO₄)₂] were obtained from Wako (Osaka, Japan). Chelex 100 sodium form was obtained from Sigma-Aldrich (St Louis, MO, USA). 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and 3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide (M4PO) were obtained from Tokyo Chemical Industry (Tokyo, Japan). GlutaMAX was purchased from Life Technologies (Carlsbad, CA, USA). All other chemicals were of the highest quality available.

Experimental setting of NTP. The NTP device, classified as plasma jet-type, was applied with a sinusoidal alternative current of 9 kV, transformed from a 100-V to 60-Hz commercial power line^{16–19}. The distance from the bottom of the plasma head round window to the top surface of a 96-well plate was fixed as 10 mm. The flow rate of argon gas was set at 2 standard liters/min.

Detection of •OH with EPR spin trapping. 100 mM M4PO or 2 mM DMPO in 50 mM phosphate buffer (pH 7.4) was directly exposed to NTP for 2 min in the presence of tetrachloroaurate (1–100 μM) or a vehicle in 96-well plates (375 μL; Thermo Fisher Scientific, Waltham, MA, USA). When evaluating the antioxidative compounds, Chelex-treated water (vehicle) or an antioxidant at the indicated concentration was irradiated simultaneously in the same well. Each sample was immediately transferred to a disposable flat quartz cell (Radical Research, Tokyo, Japan), as previously described^{16–19}. The EPR settings were as follows: microwave power, 4 mW; frequency, 9.425 GHz; central magnetic field, 340 mT; sweep width, 15 mT;

amplitude, 1 · 500; sweep time, 2 min. EPR signals were obtained using an FR-30 ESR Spectrometer equipped with ES-WINAP ESR SYSTEM v2.2.7.19. software (JEOL, Tokyo, Japan). When the signal was over-ranged, the amplitude was decreased to optimize the setting.

The effect of metal ions on lipid peroxidation using TBARS. PC was dispersed at 2.55 mM in phosphate buffer (50 mM, pH 7.4) in Chelex-treated milli-Q water, as previously described¹⁹. The PC suspension was prepared with extensive vortexing. The samples were then divided into three groups (untreated, argon gas 2 min, and NTP 2 min) and transferred into 96-well clear-bottom plates containing designated metal ions (100 µM) or an appropriate vehicle. To assess the antioxidative compound against tetrachloroaurate-induced oxidation, the compounds were added to the PC suspensions and transferred into 96-well plates containing a vehicle or tetrachloroaurate (10, 50, or 100 µM). TBARS were determined according to the manufacturer's instructions. In brief, samples were incubated with a chromogenic reagent consisting of 2-thiobarbituric acid and acid reagent for 20 min at room temperature. The fluorometric assay was subsequently performed using Powerscan4 or Cytation5 (Biotek, Winooski, VT, USA), with malondialdehyde (MDA) as a standard.

Statistical analysis. Statistical analyses were performed using a paired *t*-test in GraphPad Prism 7 (Graphpad Software, La Jolla, CA, USA). Differences were considered significant when $p < 0.05$. The data were expressed as the mean ± SEM ($n = 3-6$) unless otherwise specified.

List Of Abbreviations

DMP0; 5,5-dimethyl-1-pyrroline-*N*-oxide

EPR; electron paramagnetic resonance

GSH; glutathione, reduced form

GSSG; glutathione, oxidized form

M4PO; 3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide

MDA; malondialdehyde

NTP; non-thermal plasma

PAL; non-thermal plasma-activated Ringer's lactate

PAM; non-thermal plasma-activated medium

PC; phosphatidylcholine

ROS; reactive oxygen species

RNS; reactive nitrogen species

TBARS; 2-thiobarbituric acid reactive substances

UV; ultraviolet

Declarations

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Conflict of interest

No potential conflict of interest was disclosed.

Author Contributions

Y.O. and S.T. designed this study. Y.O., K.S., N.I. acquired the data. Y.O., K.M. and S.T. analyzed and interpreted the data. M.H. and H.T. provided the NTP device and supervised this project. Y.O. drafted manuscript. S.T. revised manuscript. All authors approved final version of manuscript.

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Figures

Figure 1, Okazaki Y, et al.

Figure 1

Determination of phosphatidylcholine (PC)-derived 2-thiobarbituric acid-reactive substances (TBARS) after NTP exposure for 2 min in the presence of ions. (A) TBARS were significantly elevated after NEAPP irradiation for 2 min, except for chromium, iron, vanadium, and gold. Each vehicle, representing a suspension buffer according to the manufacturer's product information, is categorized by an arrow. (B) Ferric ion significantly enhanced TBARS production with a significant additional increase due to NTP in a

dose-dependent manner. (C) Vanadium ions significantly enhanced TBARS production with a significant additional increase due to NTP in a dose-dependent manner. (D) Tetrachloroaurate (III) (HAuCl₄) significantly enhanced TBARS production with a significant additional increase due to NTP in a dose-dependent manner. Data are expressed as SEM (n = 4–6). (*p < 0.05, **p < 0.01, ***p < 0.001 vs NTP without metal ions, #p < 0.05, ##p < 0.01, ###p < 0.001 vs Ar gas without metal ions, †p < 0.05, ††p < 0.01, †††p < 0.001 vs argon gas for 2 min with the same concentrations of metal ions) (U; untreated, Ar; argon gas, P; NTP)

Figure 2, Okazaki Y, et al.

Figure 2

Tetrachloroaurate (HAuCl_4) yields M4PO-X. (A) The hydroxyl radical ($\bullet\text{OH}$) was generated by NTP exposure for 2 min. M4PO-OH disappeared at 25 μM of HAuCl_4 . (B) A significant yield of M4PO-X was obtained from 5 μM of HAuCl_4 . NTP irradiation for 2 min in the presence of 75 and 100 μM of HAuCl_4 significantly decayed the M4PO-X spin adduct. The representative EPR spectra are shown. ($\bullet$ indicates the M4PO-OH signal; $\bullet$ indicates the M4PO-H signal; $\boxtimes$ indicates the M4PO-X signal; ∇ indicates the manganese signal). Data are expressed as SEM ($n = 4-6$). (* $p < 0.05$, ** $p < 0.01$ vs NTP without HAuCl_4 , # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs Ar gas without HAuCl_4 , † $p < 0.05$ vs argon gas for 2 min with the same concentration of HAuCl_4)

Figure 3, Okazaki Y, et al.

Figure 3

GSH and GSSG significantly attenuate tetrachloroaurate (HAuCl_4)-induced oxidation in EPR. (A) GSH significantly attenuated HAuCl_4 -induced M4PO-X from 25 μM with Ar gas treatment. The same concentration of GSH and HAuCl_4 resulted in the disappearance of M4PO-X and M4PO-OH. Meanwhile, M4PO-X was quenched completely by the excess GSH (250 μM), while the amount of M4PO-OH was observed to be as high as that of the group with GSH in the absence of HAuCl_4 . The representative EPR

spectra are shown. (● indicates the M4PO-OH signal; ● indicates the M4PO-H signal; ☐ indicates the M4PO-X signal; ▽ indicates the manganese signal). (B) Up to 250 μ M Gly, which forms GSH with L-cysteine and L-glutamate (Glu), did not significantly suppress H_{Au}Cl₄-induced oxidation. There was no difference between the Ar gas and NTP treatments. (C) Up to 250 μ M Glu also did not significantly suppress H_{Au}Cl₄-induced M4PO-X. There was no difference between the Ar gas and NTP treatments. (D) GSSG (from 10 μ M) significantly attenuated M4PO-X in a dose-dependent manner. As observed in GSH, 100 and 250 μ M GSSG with NTP generated M4PO-OH. Data are expressed as SEM (n = 3–4). (*p < 0.05, **p < 0.01 vs NTP without H_{Au}Cl₄, #p < 0.05, ##p < 0.01 vs Ar gas without H_{Au}Cl₄)

Figure 4, Okazaki Y, et al.

Figure 4

GSH and GSSG significantly suppress tetrachloroaurate (HAuCl₄) -induced lipid peroxidation. (A, B) In the Ar gas and NTP treatments, 100 and 250 μM GSH significantly suppressed lipid peroxidation in the presence of 100 μM tetrachloroaurate. GSH (25, 50, 100, and 250 μM) significantly suppressed lipid peroxidation in the presence of 50 μM tetrachloroaurate. GSH (10, 25, 50, 100, and 250 μM) significantly suppressed lipid peroxidation in the presence of 10 μM tetrachloroaurate, while there was no significant

suppression of lipid peroxidation without tetrachloroaurate. (C, D) In the Ar gas and NTP treatments, Gly was ineffective in suppressing lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate and in its absence. (E, F) In the Ar gas and NTP treatments, Glu was ineffective in suppressing lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate and in its absence. (G, H) In the Ar gas treatment, 100 and 250 μ M GSSG significantly suppressed lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate. In the NTP treatment, 250 μ M GSSG significantly suppressed lipid peroxidation in the presence of 10, 50, and 100 μ M tetrachloroaurate. Furthermore, 100 μ M GSSG significantly suppressed lipid peroxidation in the presence of 10 μ M tetrachloroaurate. In contrast, there was no significant suppression of lipid peroxidation without tetrachloroaurate. Data are expressed as SEM (n = 3–5). (*p < 0.05, **p < 0.01, ***p < 0.001 vs NTP without antioxidant, #p < 0.05, ##p < 0.01, ###p < 0.001 vs Ar gas without antioxidant)

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