Cite this: *Toxicol. Res.*, 2018, 7, 1081

Mitochondrial toxicity induced by a thiourea gold(i) complex: mitochondrial permeability transition and respiratory deficit

Bingqiong Yu,^a Long Ma,^a Jiancheng Jin,^a Fenglei Jiang,^a Gangcheng Zhou,^a Kun Yan^{*a} and Yi Liu^{*a,b,c}

Gold(i) complexes have been widely used as antibacterial and antitumor agents because of their excellent biological activities. However, there are few reports on the study of gold(i) complexes at the subcellular level. Herein, we investigated the toxicity of a gold(i) complex (*N,N'*-disubstituted cyclic thiourea ligand) – AuTuCl – to isolated mitochondria via various methods. The results showed that AuTuCl induced mitochondrial swelling, elevated ROS generation and triggered collapse of the membrane potential, which indicated the induction of mitochondrial permeability transition (MPT). It also enhanced the permeability of H⁺ and K⁺ of the inner membrane and declined membrane fluidity, which might be the result of MPT. Moreover, AuTuCl impaired the mitochondrial respiratory chain and suppressed the activities of complexes II and IV in the respiratory chain. It also triggered the deficiency of ATP and the effusion of Cyt c, which were strictly related to respiration and apoptosis. These results indicated that AuTuCl severely affected the structure and function of mitochondria. It was proposed that MPT and impairment of the respiratory chain were responsible for the mitotoxicity of AuTuCl, thus causing energy deficiency and even apoptosis. This conceivable mechanism can serve as a clue for better understanding of the toxicology of AuTuCl.

Received 30th June 2018,
Accepted 29th August 2018

DOI: 10.1039/c8tx00169c

rsc.li/toxicology-research

Introduction

Mitochondria, which are enclosed compartments covered by a double-layer membrane, can be divided into an outer membrane, an inner membrane, and an inter-membrane cavity according to their functions. Mitochondria play essential roles in life processes. They are regarded as the cell's energy factory, which convert oxygen and nutrients into adenosine triphosphate (ATP) through oxidative phosphorylation.^{1–3} The dysfunction of mitochondria would lead to various diseases,^{4,5} which are closely related to apoptosis and tumor induction.^{6–8}

Metal-based drugs represent one of the most studied classes of anticancer chemotherapeutic agents such as platinum-based drugs.^{9–11} However, there remain some draw-

backs such as severe side effects and drug resistance, which stimulate research on other metal species. Among the non-platinum complexes, gold(i) complexes exhibit excellent drug effects.^{12–15} Under physiological conditions, hydrated Au⁺ ions are unstable toward precipitation, reduction or aerobic oxidation. These deficiencies can be avoided by applying appropriate ligands to increase their stabilities. Among the gold(i) metal complexes, gold(i) phosphine complexes are frequently investigated.^{16–19} The most well-known example is auranofin, which has been widely used as an anti-arthritis agent.^{20–22} In 2010, Yan *et al.* reported a gold(i) complex of *N,N'*-disubstituted cyclic thiourea.²³ This thiourea gold(i) complex selectively inhibited the activity of thioredoxin reductase (TrxR) and triggered apoptosis, showing excellent biological activities. The mitochondrial Trx/TrxR system is crucial for balancing cellular redox, oxidative stress and cell death.^{24,25} So we suspect that this complex also causes damage to mitochondria.

In this work, we explored the biological effects of AuTuCl on the isolated mitochondria through different methods. The results showed that AuTuCl of low concentrations induced mitochondrial swelling, reduced the mitochondrial membrane potential and enhanced ROS generation, indicating the occurrence of mitochondrial permeability transition (MPT). AuTuCl

^aState Key Laboratory of Virology & Key Laboratory of Analytical Chemistry for Biology and Medicine (MOE) College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China. E-mail: yiliuchem@whu.edu.cn; Fax: +8627-68754067; Tel: +8627-68753465

^bCollege of Chemistry and Material Sciences, Guangxi Teachers Education University, Nanning 530001, P. R. China

^cKey Laboratory of Coal Conversion and New Carbon Materials of Hubei Province, College of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, P. R. China

also altered membrane fluidity, which confirmed the occurrence of MPT. Besides, it triggered the impairment of mitochondrial respiration, reduced the production of ATP and promoted the release of Cyt *c*, which demonstrated that AuTuCl aroused mitochondrial respiration disorder. These results are expected to clarify the mechanism of the toxicity of mitochondria induced by AuTuCl.

Experimental

Materials and methods

Cyclosporin A (CsA), adenosine diphosphate (ADP), rhodamine 123 (Rh123), ruthenium red (RR), 1,6-diphenylhexa-1,3,5-triene (DPH), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), dithiothreitol (DTT), rotenone, antimycin A, EGTA and oligomycin were obtained from Sigma-Aldrich (St Louis, USA). Bovine serum albumin (BSA) was acquired from Ruji (Shanghai, China). All other reagents were of analytical reagent grade and used without further purification. CsA, oligomycin, and rotenone were dissolved in EtOH. DPH was dissolved in THF. Other solutions were dissolved in aseptic di-water unless otherwise stated.

Characterization of complex AuTuCl

The synthesis and characterization of AuTuCl were based on the literature reports.^{23,26} The chemical structure of AuTuCl is shown in Fig. 1. AuTuCl was dissolved in DMSO and then diluted into an aqueous solution. The final volume of DMSO was no more than 3%.

Animals

Female Wistar rats (150 g–250 g) were purchased from Hubei Center for Disease Control and Prevention (Wuhan, China). The rats were bred in monitor houses, and they could get food and water freely. Purification of the rat liver mitochondria was performed following the rules of Guidelines of the China Animal Welfare Legislation, which was recognized by the Committee on Ethics in the Care and Use of Laboratory Animals and approved by the Committee on Ethics in the Care and Use of Laboratory Animals of the College of Life Sciences, Wuhan University.

Fig. 1 The chemical structure of AuTuCl.

Formulas for solutions

The buffers used in this paper were prepared according to the following formula: all the solutions were adjusted to pH values of 7.20–7.40. Solution A (0.07 M sucrose, 0.02 M HEPES, 0.22 M mannitol, 2 mM Tris, 1 mM EDTA); media B (0.07 M sucrose, 0.22 M mannitol, 5 mM Tris, 1 mM EDTA); buffer C (0.07 M sucrose, 0.22 M mannitol, 1 mM EDTA); solution D (0.2 M sucrose, 1 mM Tris, 1 mM Mops, 10 μ M EGTA, 1 mM Na_3PO_4 , 3 μ g mL^{-1} oligomycin, 5 mM succinate, 2 μ M rotenone); media E (0.1 M sucrose, 10 mM Tris, 1 mM EDTA, 50 mM KCl, 2 mM MgCl_2 , 10 mM Mops, 10 mM KH_2PO_4 , 2 μ M rotenone); solution F (175 mM KCl, 10 mM Tris, 2 μ M rotenone); buffer H (0.135 M CH_3COOK , 5 mM HEPES, 0.1 mM EGTA, 2 μ M rotenone, 1 μ g mL^{-1} valinomycin, 0.2 mM EDTA); solution K (0.135 M KNO_3 , 5 mM HEPES, 0.1 mM EGTA, 0.2 mM EDTA).

Isolation of mitochondria

The liver mitochondria were separated and purified through gradient centrifugation.^{27,28} The protocols were performed at 0–4 °C. The rat liver was quickly sheared and washed with solution A three times. About 3 g mince was suspended in 30 mL of 0.4% BSA solution A and homogenized in a Dounce Tissue Grinder (WHEATON). Then, the homogeneous fluid was centrifuged at 3000g for 5 min to get rid of the debris and mustard tissues. The supernatant was collected and centrifuged at 10 000g for 3 min. The precipitate was collected and washed with solution A and then with solution B. Finally, the enriched mitochondria were resuspended in buffer C for further experiments. The mitochondrial protein concentration was determined by the biuret method using BSA as a standard.²⁹

Mitochondrial swelling and the permeability of H^+ and K^+

Mitochondrial swelling was measured by tracking the absorbance at 540 nm at 25 °C.³⁰ Mitochondria (0.5 mg mL^{-1}) were suspended in solution D and different concentrations of complex AuTuCl were added to incubate for 30 min. The spectra were recorded on a multimode plate reader VICTOR™ X5 (PE, USA) or a UV-6100PC UV-Vis spectrophotometer (MAPADA, China). For the H^+ permeabilization experiment, buffer H was used. For the K^+ permeabilization test, solution K was introduced.³¹ The detection method was similar to that of the swelling experiment.

Mitochondrial membrane potential

The mitochondrial membrane potential was measured by detecting the fluorescence intensity of Rh123 ($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 525 \text{ nm}$).³² Mitochondria (0.5 mg mL^{-1}) and Rh123 (200 nM) were dispersed in solution D. Then, different concentrations of AuTuCl were added and examined.

Mitochondrial membrane fluidity

The mitochondrial membrane fluidity was evaluated by measuring the alteration of the anisotropy of DPH-labelled

mitochondria.^{33,34} A DPH (dissolved in THF) solution was prepared just before use with the final concentration of 2 μM . The DPH solution was added to the stirred mitochondrial solution (0.5 mg mL^{-1} , solution D). After incubation for 10 min, different concentrations of AuTuCl were injected and monitored through a LS55 fluorescence spectrophotometer (PE, USA). The anisotropy (r) was monitored at 454 nm ($\lambda_{\text{ex}} = 362 \text{ nm}$).

Mitochondrial respiration

The amount of oxygen consumption was measured with a Clark Oxygen Electrode (Hansatech Instruments, Norfolk, UK) at 25 °C.³⁵ Mitochondria (1 mg mL^{-1}) were added to media E and incubated for 3 min with the isolation of air. Then different concentrations of AuTuCl were added, and initiators were injected immediately. State 2/4 was triggered by adding sodium succinate (5 mM). State 3 was energized by the addition of succinate (5 mM) and ADP (1 mM). For the uncoupled respiration, 30 μM DNP was added.

Measurement of ROS generation

The increase of reactive oxygen species was gauged through examining the fluorescence intensity of DCFH-DA.³⁶ Briefly, mitochondria (0.5 mg mL^{-1}) were cultivated with different concentrations of AuTuCl in buffer E (with the addition of 5 mM succinate sodium) for 30 min at room temperature. Then DCFH-DA (500 nM) was introduced to incubate for another 15 min with the suspension. The green fluorescence signals were monitored using a flow cytometer (BD, C6) or a multimode plate reader VICTOR™ X5 (PE, USA).

Determination of Cyt c

The release of Cyt c was measured by using a Cyt c ELISA Kit. Mitochondria (0.5 mg mL^{-1}) were suspended in media D (0.5 mL) and incubated with AuTuCl for 30 min at 25 °C. The following operations were performed according to the manufacturer's protocol.

Assessment of ATP

Mitochondria (1 mg mL^{-1}) were incubated with AuTuCl in solution D. After 30 min, the suspension was centrifuged at 13 000g at 4 °C for 5 min to obtain the deposit. Then the remaining operations were performed using a commercial ATP Assay Kit (Beyotime, S0026).

Measurement of respiratory chain complex II

The activity of complex II was measured *via* quantitative detection of the reduction of MTT.³⁷ Mitochondria (0.5 mg mL^{-1}) were suspended in solution D, and different concentrations of AuTuCl were added to incubate for 30 min at 25 °C. Then the MTT solution (4% dissolved in PBS) was added to cultivate for another 30 min. Finally, 10% SDS was added into the suspension to dissolve the purple formazan crystals. The absorbance at 490 nm was collected with a multimode plate reader.

Assessment of respiratory chain complex IV

The activity of complex IV was determined by using a commercial Rat Cox ELISA Kit. Briefly, 500 μL mitochondria (0.5 mg mL^{-1}) were incubated with various concentrations of AuTuCl for 30 min at room temperature. Then the following procedures were strictly carried out according to the instructions of the kit.

Mitochondrial ultrastructure

The ultrastructure of mitochondria was studied by transmission electron microscopy (TEM). The mitochondria were incubated with AuTuCl at room temperature for 30 min in solution C. Then the suspension liquid was centrifuged at 10 000g for 5 min to obtain the substrate. The precipitate was fixed with 2.5% glutaraldehyde PBS buffer for at least 30 min, post-fixed with 1% osmium tetroxide and dehydrated.

Results and discussion

Mitochondrial swelling and the permeability of H^+ and K^+

Swelling is one of the signs of mitochondrial dysfunction.³⁸ When the mitochondria suffer from oxidative stress, Ca^{2+} overfilling or other stimulations, small molecules such as water enter into the mitochondrial matrix, squeeze the inner membrane and cause vacuolization. Then the mitochondria become transparent and swollen, and their absorbance decreases. As shown in Fig. 2, in the control group, the absorbance at 540 nm remained unchanged indicating the activity and integrity of mitochondria, while in the AuTuCl exposed group, the absorbance declined in a time-dependent manner. When the drug concentration increased to 15 μM , the absorbance dropped to 75% compared to the control, indicating that the mitochondria were severely swollen. As reported in the literature, other materials induced mitochondrial swelling at concentrations up to 100 μM ,³⁹ but AuTuCl caused mitochondrial swelling at low concentrations. These facts manifested that AuTuCl is more effective at damaging mitochondria.

During the past decade, the mitochondrial degeneration has been ascribed to the opening of the mitochondrial permeability transition pore (MPTP),⁴⁰ which could permeate

Fig. 2 Mitochondrial swelling induced by different concentrations of AuTuCl. c (AuTuCl)/ μM a–f: 0, 2, 5, 7, 10, and 15.

solutes up to 1.5 kDa. In order to ascertain the swelling mechanism, different inhibitors were applied to the test such as DTT, CsA, EDTA, ADP, EGTA, and RR. CsA is a traditional inhibitor for the MPTP through binding with cyclophilin D⁴¹ which mainly locates at the mitochondrial matrix. When the MPT occurs, it translocates to the inner membrane of the mitochondria so as to elevate the permeability of the inner membrane. As a result, the opening of the MPTP is repressed. ADP is another effective agent to inhibit MPTP opening. It interacts with the adenine nucleoside transporter, making it difficult to change the conformation of MPTP. EDTA is a metal chelating agent which could effectively chelate metal ions to prevent the injury of heavy metal ions. RR is a non-competitive inhibitor of the Ca²⁺ unidirectional transporter.⁴² It prevents the efflux of Ca²⁺ to the matrix, thus suppressing the Ca²⁺-dependent mitochondrial damage. DTT is a reducing agent. It is able to oxidize the S-sites of the inner mitochondrial membrane, thereby protecting the function of the S-sites.⁴³ As shown in Fig. 3a and b, EDTA, EGTA, RR and ADP could hardly restrain the mitochondrial swelling. However, CsA reversed the swelling aroused by AuTuCl which supported the opening of the MPTP. DTT also totally inhibited the swelling, indicating the possible interaction between the sulfhydryl-containing proteins and AuTuCl, thus leading to the disorder of protein. These protection experiments demonstrated that the swelling induced by AuTuCl was probably due to the MPT and the interaction with the S-site protein on the membrane.

Mitochondrial inner membranes are highly selective for ions, especially H⁺ and K⁺. When the mitochondrial inner membrane is altered, the penetration of H⁺ and K⁺ becomes disordered, thus leading to the mitochondrial swelling and collapse of the membrane potential. Therefore, it is necessary to examine the ability to penetrate H⁺ and K⁺ for detecting the mitochondrial membrane integrity.⁴⁴ For measuring the permeability of K⁺, driven by the potential difference, K⁺ is transported from the inner membrane to the matrix. So under normal conditions, the mitochondria become swollen and the absorbance at 540 nm declines slowly. When the infiltration of H⁺ of the intima is examined, valinomycin which is a lipid-soluble K⁺ transport carrier is added to eliminate the effect of K⁺ on the potential. H⁺ on the outside binds with CH₃COO⁻, forming CH₃COOH which can have access to the matrix. So the H⁺ enters into the matrix in another form to reduce the proton concentration gradient and the potential difference. Finally, the mitochondria become swollen and the absorbance drops. Fig. 3c and d show that in AuTuCl treated mitochondria, the slope of the straight line decreased more sharply compared to the control ones, which indicated that AuTuCl promoted K⁺ and H⁺ permeabilization of the inner membrane and changed the structure of the inner membrane.

The collapse of mitochondrial membrane potential

The reduction of the membrane potential is another sign of MPT. The membrane potential was determined by using the

Fig. 3 MPT mechanism induced by complex AuTuCl. (a) Different protective agents were pre-treated with the mitochondria for 10 min. a: control, b: 15 μ M AuTuCl, c: 15 μ M AuTuCl + 40 μ M ADP, d: 15 μ M AuTuCl + 20 μ M CsA, e: 15 μ M AuTuCl + 40 μ M EDTA. (b) Different protective agents were pre-treated with the mitochondria for 10 min. a: control, b: 15 μ M AuTuCl, c: 15 μ M AuTuCl + 20 μ M RR, d: 15 μ M AuTuCl + 20 μ M DTT, e: 15 μ M AuTuCl + 20 μ M EGTA. (c) The permeability of H⁺ to the mitochondrial inner membrane. c (AuTuCl)/ μ M a–e: 0, 2, 5, 10, and 15. (d) The permeability of K⁺ to the mitochondrial inner membrane. c (AuTuCl)/ μ M a–f: 0, 5, 8, 10, 15, and 20.

fluorescent probe Rh123.^{37,45} The mitochondrial inner membrane has a negative internal potential difference. Rh123 is a lipid-soluble positive cation probe, which can penetrate the inner membrane and enter into the mitochondrial matrix due to the membrane potential gradient. When the mitochondria are damaged, the potential difference cannot be maintained, and Rh123 is released from mitochondria. So the fluorescence intensity gets rebounded. As shown in Fig. 4a, when different concentrations of AuTuCl were added, the fluorescence intensity recovered in a dose-dependent manner. The results pointed out that AuTuCl triggered the collapse of the membrane potential. When the mitochondrial potential declines, the infiltration of H^+ and K^+ by the inner membrane is promoted, and also causes other changes such as disorder of the respiratory chain.

The elevation of ROS generation

When the MPT occurs, it is often followed by the generation of ROS (including hydroxyl radical, superoxide anion and hydrogen peroxide) which have been widely believed to play an essential role in apoptosis. They are mainly generated by the mitochondrial respiratory chain complexes especially complex I and complex III. The measurement of ROS is indispensable for exploring the mitochondrial dysfunction. DCFH-DA, which can be attacked by ROS to become fluorescent DCF as the DCFH-DA displays no fluorescence, was applied to measure the amount of ROS. As shown in Fig. 4b, the level of ROS generation increased moderately, in a concentration-dependent manner. Due to ROS generation, the mitochondria encountered a series of oxidative stress, thus leading to the mitochondrial dysfunction.

The decrease of membrane fluidity

Since AuTuCl induced MPT induction, it has been established that the change of membrane fluidity has a direct impact on MPT, and then we measured the membrane fluidity. The membrane fluidity refers to the motion state of phospholipids and membrane proteins which regulate the mitochondrial sequence.^{46,47} It (critical to function and adaption to various outside stimuli) would affect the mitochondrial energy meta-

bolism. It has been reported that the increase of membrane fluidity could strengthen the function of mitochondria. Therefore, the detection of membrane fluidity is critically important to measure the mitochondrial function. The membrane fluidity can be determined by measuring the variation of fluorescence anisotropy (r) of the fluorescent dye DPH, which is usually applied to reflect the fluidity of the hydrophobic area of the membrane. The anisotropy value is negatively correlated with membrane fluidity.³³ As shown in Fig. 5, the anisotropy (r) value of the blank set remained stable, while in the AuTuCl treated ones, the anisotropy (r) value increased sharply. The gain of anisotropy (r) was probably due to the energy transduction between chromophores or slowing down of Brownian motion. The results indicated that AuTuCl slowed down the membrane fluidity and destructed the membrane structure. When the membrane fluidity is altered, some enzyme proteins are blocked, oxidative phosphorylation is reduced, and energy supply is insufficient. As a result, the change of membrane fluidity would lead to other mitochondrial impairment. These data indicated that MPT induction was caused by AuTuCl possibly through the decrease of membrane fluidity.

The deficit in mitochondrial respiration

Mitochondrial respiration is critically essential for cell survival. The experiment started with the second respiration

Fig. 5 The decrease of mitochondrial membrane fluidity detected by DPH (2 μ M) c (AuTuCl)/ μ M a–e: 0, 2, 5, 10, and 15.

Fig. 4 Mitochondrial membrane function alteration induced by AuTuCl. (a) The decline of the mitochondrial membrane potential detected by Rh123 (400 nM). (b) The elevation of ROS generation measured with DCFH-DA (500 nM).

chain, as rotenone was added to the solution, which could inhibit the main respiration chain.⁴⁸ State 2 is the respiration before the addition of ADP. State 4 is defined as the state with no ATP (addition of oligomycin). State 2 usually functions the same way as state 4 which is usually used for both states.^{49,50} We preferred to use state 4 because it is physiologically more meaningful. State 3 is the state when sodium succinate and ADP are simultaneously added and they feature in the respiration chain and the synthesis of ATP. The ratio of the respiration rate of state 3 to the respiration rate of state 4 is defined as the respiratory control ratio (RCR).^{51,52} The RCR value intuitively shows the efficiency of the oxidative phosphorylation of mitochondria. As shown in Fig. 6a, in the presence of AuTuCl, the respiration in state 4 increased intensely. However, the respiration rate of state 3 and the uncoupled state decreased mildly, which indicated that complex AuTuCl impaired the mitochondrial respiratory chain severely. As depicted in Fig. 6b, the RCR value was 3.36 manifesting the intact mitochondria in the control group. However, the RCR value reduced acutely in the presence of AuTuCl reflecting that AuTuCl significantly reduced mitochondrial oxidative phosphorylation efficiency and respiratory function. It has been shown that there is a relationship between membrane fluidity and respiratory chain complexes, as well as the electron transfer chain. As displayed in our results, the membrane fluidity reduced and the respiratory chain was destroyed. So it was appropriate to declare that the change of membrane fluidity is one of the factors affecting the respiratory chain.

The activity of complexes II and IV

Complex II, also called succinate dehydrogenase, can receive electrons from succinic acid and pass it to coenzyme Q. Succinate dehydrogenase can reduce yellow MTT to a purple crystal.⁵³ By quantitatively detecting the content of the purple crystal, the activity of complex II can be reflected. As shown in Fig. 6c, the absorbance at 490 nm dropped 10% even at a low concentration (5 μM) in AuTuCl co-treated mitochondria. When the dose increased to 12.5 μM , the activity of complex II reduced to about 50% in contrast to the control. The results showed that the activity of complex II was severely affected in the presence of AuTuCl.

Complex IV (Cyt *c* oxidase) lies at the end of the cytochrome system in cellular respiration. This enzyme transfers the electrons of the respiratory substrate directly to the molecular oxygen through the cytochrome system. Fig. 6d indicates that the activity of complex IV also declined in the presence of AuTuCl. These data showed that AuTuCl inhibited the activities of complexes II and IV, which proved that AuTuCl indeed disturbed the mitochondrial respiration.

The measurement of Cyt *c* and ATP

Detecting the amount of ATP serves as a straightforward proof to monitor the function of mitochondria. As mentioned above, the reduction of membrane fluidity may result in the ATP content decline. Moreover, the decrease of the ATP amount is related to MPT.⁵⁴ As displayed in Fig. 7a, the amount of ATP

Fig. 6 The effect of mitochondrial respiration in the presence of complex AuTuCl. (a) Respiration in state 3 (blue line) and the uncoupled state (red line) decreased. Respiration in state 4 (black line) increased. (b) The respiratory control ratio (RCR) declined. (c) The activities of respiratory chain complex II. (d) The activities of respiratory chain complex IV.

Fig. 7 (a) The effects of AuTuCl on the release of Cyt c. (b) The mitochondrial ATP generation induced by AuTuCl.

produced by mitochondria declined in a concentration-dependent manner in AuTuCl treated groups. When the concentration of AuTuCl increased to 12 μM, the amount of ATP decreased to 50% compared with the control. The fall of the ATP level indicated the decrease of the oxidative phosphorylation of mitochondria, which also supported the damage caused to respiration. The occurrence of MPT is often accompanied by the release of Cyt *c* which is a crucial electron transporter in the respiratory chain. The release of Cyt *c* is often coupled with caspase-dependent apoptosis.⁵⁵ Fig. 7b shows that in the AuTuCl treated mitochondria, the release of Cyt *c* elevated, which may induce caspase-dependent apoptosis.

Mitochondrial ultrastructure

The ultrastructure of mitochondria was examined by transmission electron microscopy (TEM). TEM was an evidential method to verify whether the mitochondria were damaged. As shown in Fig. 8a, the normal mitochondria isolated from rat liver were well maintained as the outer and inner membranes were integrated. The matrix was compact and the ridges of the inner membrane were clear. When the mitochondria were incubated with 5 μM AuTuCl, some mitochondria become swollen and transparent. The ridges of the inner membrane were blurred. When the dose concentration increased to 20 μM, more vacuoles appeared, showing irregular patterns and a large volume. Moreover, the matrix electron density and the inter membrane space decreased, the ridges of the inner membrane disappeared and cristae ruptured (Fig. 8b–d). These clues indicated that AuTuCl severely injured the mitochondrial structure. The alteration of the ultrastructure also confirmed the impairment of the mitochondrial function. The possible mechanism of mitochondrial toxicity induced by AuTuCl is depicted in Fig. 9.

Fig. 8 The mitochondrial ultrastructure observed by TEM. c (AuTuCl)/μM a–d: 0, 5, 10, and 20.

Fig. 9 Proposed mechanisms of mitochondrial dysfunction induced by AuTuCl.

Conclusions

In summary, the present data showed that the gold(I) complex AuTuCl induced mitochondrial swelling, which could be restrained by DTT and CsA indicating that AuTuCl triggered MPT. Moreover, it decreased the membrane fluidity, which was probably a possible mechanism of MPT occurrence. AuTuCl also increased the permeability of H^+ and K^+ , decreased the membrane potential, and increased ROS generation which were also the result of MPT. On the other hand, AuTuCl impaired mitochondrial respiration, inhibited the activity of complexes II and IV in the respiratory chain, triggered the deficit of ATP and the efflux of Cyt c, which demonstrated that AuTuCl severely affected mitochondrial respiration and metabolism. Both the MPT and the impairment of the respiratory chain were responsible for the toxicity of AuTuCl. Our data provided us auxiliary information for better understanding the mechanism of toxicity of the gold(I) complexes.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Science Foundation of China (No. 21401144), Nature Science Foundation of Hubei Province (No. 2013CFB236) and Guangxi Science and Technology Project (GuiKeAD17195081) and Bagui Scholar Program of Guangxi Province (2016).

Notes and references

- 1 Z. H. Wang, C. Clark and E. R. Geisbrecht, Analysis of mitochondrial structure and function in the *Drosophila* larval musculature, *Mitochondrion*, 2016, **26**, 33–42.
- 2 P. Gazzotti, Mitochondria: a general survey, *Top. Bioelectrochem. Bioenerg.*, 1980, **3**, 149–190.
- 3 C. D. Berdanier, Introduction to mitochondria, *Oxid. Stress Dis.*, 2005, **16**, 1–64.
- 4 K. John, V. Alla, C. Meier and B. M. Puetzer, GRAMD4 mimics p53 and mediates the apoptotic function of p73 at mitochondria, *Cell Death Differ.*, 2011, **18**, 874–886.
- 5 L. Kong, X. Zhou, Y. Wu, Y. Wang, L. Chen, P. Li, S. Liu, S. Sun, Y. Ren, M. Mei, X. Wang and L. Zhang, Targeting HOTAIR induces mitochondria related apoptosis and inhibits tumor growth in head and neck squamous cell carcinoma in vitro and in vivo, *Curr. Mol. Med.*, 2015, **15**, 952–960.
- 6 G. Paradies, V. Paradies, F. M. Ruggiero and G. Petrosillo, Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease, *World J. Gastroenterol.*, 2014, **20**, 14205–14218.
- 7 B. Osborne, N. L. Bentley, M. K. Montgomery and N. Turner, The role of mitochondrial sirtuins in health and disease, *Free Radical Biol. Med.*, 2016, **100**, 164–174.
- 8 X. R. Liu, Z. G. Jia and J. H. Chen, Enhanced sampling of intrinsic structural heterogeneity of the BH_3 -only protein binding interface of Bcl-xL, *J. Phys. Chem. B*, 2017, **121**, 9160–9168.
- 9 N. J. Wheate, S. Walker, G. E. Craig and R. Oun, The status of platinum anticancer drugs in the clinic and in clinical trials, *Dalton Trans.*, 2010, **39**, 8113–8127.
- 10 S. Dilruba and G. V. Kalayda, Platinum-based drugs: past, present and future, *Cancer Chemother. Pharmacol.*, 2016, **77**, 1103–1124.
- 11 J. Kim, S. Pramanick, D. Lee, H. Park and W. J. Kim, Polymeric biomaterials for the delivery of platinum-based anticancer drugs, *Biomater. Sci.*, 2015, **3**, 1002–1017.
- 12 R. W. Y. Sun and C. M. Che, The anti-cancer properties of gold(III) compounds with dianionic porphyrin and tetradentate ligands, *Coord. Chem. Rev.*, 2009, **253**, 1682–1691.
- 13 I. Ott, On the medicinal chemistry of gold complexes as anticancer drugs, *Coord. Chem. Rev.*, 2009, **253**, 1670–1681.
- 14 R. W. Sun, D. L. Ma, E. L. Wong and C. M. Che, Some uses of transition metal complexes as anti-cancer and anti-HIV agents, *Dalton Trans.*, 2007, **21**, 4884–4892.
- 15 T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang and C. M. Che, Chemical biology of anticancer gold(III) and gold(I) complexes, *Chem. Soc. Rev.*, 2015, **44**, 8786–8801.
- 16 V. Andermark, K. Goeke, M. Kokoschka, M. A. Abu EL Maaty, C. T. Lum, T. Zou, R. W. Sun, E. Aguilo, L. Oehninger, L. Rodriguez, H. Bunjes, S. Woelfl, C. M. Che and I. Ott, Alkynyl gold(I) phosphane complexes: evaluation of structure-activity-relationships for the phosphane ligands, effects on key signaling proteins and preliminary in-vivo studies with a nanoformulated complex, *J. Inorg. Biochem.*, 2016, **160**, 140–148.
- 17 J. Fernandez-Gallardo, B. T. Elie, T. Sadhukha, S. Prabha, M. Sanau, S. A. Rotenberg, J. W. Ramos and M. Contel, Heterometallic titanium-gold complexes inhibit renal cancer cells in vitro and in vivo, *Chem. Sci.*, 2015, **6**, 5269–5283.
- 18 R. Galassi, A. Burini, S. Ricci, M. Pellei, M. P. Rigobello, A. Citta, A. Dolmella, V. Gandin and C. Marzano, Synthesis and characterization of azolate gold(I) phosphane complexes as thioredoxin reductase inhibiting antitumor agents, *Dalton Trans.*, 2012, **41**, 5307–5318.
- 19 B. Kemper, M. V. Groening, V. Lewe, D. Spitzer, T. Otremba, N. Stergiou, D. Schollmeyer, E. Schmitt, B. J. Ravoo and P. Besenius, Reversible covalent and supramolecular functionalization of water-soluble gold(I) complexes, *Chem. – Eur. J.*, 2017, **23**, 6048–6055.
- 20 T. Marzo, D. Cirri, C. Gabbiani, T. Gamberi, F. Magherini, A. Pratesi, A. Guerri, T. Biver, F. Binacchi, M. Stefanini, A. Arcangeli and L. Messori, Auranofin, Et_3PAuCl , and Et_3PAuI are highly cytotoxic on colorectal cancer cells: A

- chemical and biological study, *ACS Med. Chem. Lett.*, 2017, **8**, 997–1001.
- 21 F. Radenkovic, O. Holland, J. J. Vanderlelie and A. V. Perkins, Selective inhibition of endogenous antioxidants with auranofin causes mitochondrial oxidative stress which can be countered by selenium supplementation, *Biochem. Pharmacol.*, 2017, **146**, 42–52.
 - 22 H. Huang, Y. Liao, N. Liu, J. Cai, X. Wang and J. Liu, Novel use of clinical drugs: deubiquitinase inhibitor auranofin and disulfiram show synergistic anti-tumor effects in vitro and in vivo, *Cancer Cell Microenviron.*, 2016, **3**, 1191–1194.
 - 23 K. Yan, C. N. Lok, K. Bierla and C. M. Che, Gold(I) complex of N,N'-disubstituted cyclic thiourea with in vitro and in vivo anticancer properties - potent tight-binding inhibition of thioredoxin reductase, *Chem. Commun.*, 2010, **46**, 7691–7693.
 - 24 J. Lu and A. Holmgren, Thioredoxin system in cell death progression, *Antioxid. Redox Signaling*, 2012, **17**, 1738–1747.
 - 25 A. L. Dafre, J. Goldberg, T. Wang, D. A. Spiegel and P. Maher, *Free Radicals Biol. Med.*, 2015, **89**, 8–19.
 - 26 G. C. Zhou and K. Yan, *Study on design, synthesis and anticancer properties of novel gold(I)-thiourea complexes*, Wuhan University, 2016.
 - 27 C. D. Williamson, D. S. Wong, P. Bozidis, A. P. Zhang and A. M. Colberg-Poley, Isolation of endoplasmic reticulum, mitochondria, and mitochondria-associated membrane and detergent resistant membrane fractions from transfected cells and from human cytomegalovirus-infected primary fibroblasts, *Curr. Protoc. Cell Biol.*, 2015, **68**, 1–33.
 - 28 P. Dong, J. H. Li, S. P. Xu, X. J. Wu, X. Xiang, Q. Q. Yang, J. C. Jin, Y. Liu and F. L. Jiang, Mitochondrial dysfunction induced by ultra-small silver nanoclusters with a distinct toxic mechanism, *J. Hazard. Mater.*, 2016, **308**, 139–148.
 - 29 A. G. Gornall, C. J. Bardawill and M. M. David, Determination of serum proteins by means of the biuret reaction, *J. Biol. Chem.*, 1949, **177**, 751–766.
 - 30 J. Li, W. Yu, X. T. Li, S. H. Qi and B. Li, The effects of propofol on mitochondrial dysfunction following focal cerebral ischemia-reperfusion in rats, *Neuropharmacology*, 2014, **77**, 35–368.
 - 31 X. Y. Fan, L. Yuan, C. Wu, Y. J. Liu, F. L. Jiang, Y. J. Hu and Y. Liu, Mitochondrial toxicity of organic arsenicals: membrane permeability transition pore opening and respiratory dysfunction, *Toxicol. Res.*, 2018, **7**, 191–200.
 - 32 A. Baracca, G. Sgarbi, G. Solaini and G. Lenaz, Rhodamine 123 as a probe of mitochondrial membrane potential: evaluation of proton flux through F₀ during ATP synthesis, *Biochim. Biophys. Acta*, 2003, **1606**, 137–146.
 - 33 J. Eckmann, L. E. Clemens, S. H. Eckert, S. Hagl, L. Yu-Taeger, T. Bordet, R. M. Pruss, W. E. Muller, K. Leuner, H. P. Nguyen and G. P. Eckert, Mitochondrial membrane fluidity is consistently increased in different models of huntington disease: restorative effects of olesoxime, *Mol. Neurobiol.*, 2014, **50**, 107–118.
 - 34 J. Hu, P. Liang, X. Shi and X. Gao, Effects of insecticides on the fluidity of mitochondrial membranes of the diamond-back moth, *plutella xylostella*, resistant and susceptible to avermectin, *J. Insect Sci.*, 2008, **8**, 3.
 - 35 J. P. Monteiro, P. J. Oliveira, A. J. M. Moreno and A. S. Jurado, Disruption of hepatic mitochondrial bioenergetics is not a primary mechanism for the toxicity of methoprene – Relevance for toxicological assessment, *Chemosphere*, 2008, **72**, 1347–1354.
 - 36 L. Yuan, J. Q. Zhang, Y. J. Liu, J. Zhao, F. L. Jiang and Y. Liu, Indium(III) induces isolated mitochondrial permeability transition by inhibiting proton influx and triggering oxidative stress, *J. Inorg. Biochem.*, 2017, **177**, 17–26.
 - 37 L. Ma, J. Y. Liu, J. X. Dong, Q. Xiao, J. Zhao and F. L. Jiang, Toxicity of Pb²⁺ on rat liver mitochondria induced by oxidative stress and mitochondrial permeability transition, *Toxicol. Res.*, 2017, **6**, 822–830.
 - 38 A. A. Gerencser, J. Doczi, B. Töröcsik, E. Bossy-Wetzel and V. Adam-Vizi, Mitochondrial swelling measurement in situ by optimized spatial filtering: astrocyte-neuron differences, *Biophys. J.*, 2008, **95**, 2583–2598.
 - 39 J. Zhao, J. C. Jin, Z. Q. Zhou, C. F. Xia, X. G. Yang, F. L. Jiang, J. Dai and Y. Liu, High concentration of gadolinium ion modifying isolated rice mitochondrial biogenesis, *Biol. Trace Elem. Res.*, 2013, **156**, 308–315.
 - 40 A. Canta, E. Pozzi and V. A. Carozzi, Mitochondrial dysfunction in chemotherapy-induced peripheral neuropathy (CIPN), *Toxics*, 2015, **3**, 198–223.
 - 41 L. Marroquin, R. Swiss and Y. Will, Identifying compounds that induce opening of the mitochondrial permeability transition pore in isolated rat liver mitochondria, *Curr. Protoc. Toxicol.*, 2014, **60**, 1–17.
 - 42 M. Zoratti and I. Szabò, The mitochondrial permeability transition, *Biochim. Biophys. Acta*, 1995, **1241**, 139–176.
 - 43 A. J. Kowaltowski, R. F. Castilho and A. E. Vercesi, Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca²⁺ is dependent on mitochondrial-generated reactive oxygen species, *FEBS Lett.*, 1996, **378**, 150–152.
 - 44 J. I. Gao, M. Wu, X. Wang, Y. Z. Zhang, F. L. Jiang, Y. Liu and J. Dai, Membrane permeability transition and dysfunction of rice mitochondria effected by Er(III), *J. Membr. Biol.*, 2015, **248**, 39–46.
 - 45 L. Yuan, T. Gao, H. He, F. L. Jiang and Y. Liu, Silver ion-induced mitochondrial dysfunction via a nonspecific pathway, *Toxicol. Res.*, 2017, **6**, 621–630.
 - 46 F. Ricchelli, S. Gobbo, G. Moreno and C. Salet, Changes of the fluidity of mitochondrial membranes induced by the permeability transition, *Biochemistry*, 1999, **38**, 9295–9300.
 - 47 L. Ma, J. X. Dong, C. Wu, X. Y. Li, J. Chen, H. Zhang and Y. Liu, Spectroscopic, polarographic, and microcalorimetric studies on mitochondrial dysfunction induced by ethanol, *J. Membr. Biol.*, 2017, **250**, 195–204.
 - 48 O. Sobotka, Z. Drahota, O. Kucera, R. Endlicher, H. Rauchova and Z. Cervinkova, The effect of α -tocopheryl succinate on succinate respiration in rat liver mitochondria, *Physiol. Res.*, 2015, **64**, 609–615.

- 49 A. C. Wei, M. A. Aon, B. O'Rourke, R. L. Winslow and S. Cortassa, Mitochondrial energetics, pH regulation, and ion dynamics: a computational-experimental approach, *Biophys. J.*, 2011, **100**, 2894–2903.
- 50 B. Korzeniewski, 'Idealized' state 4 and state 3 in mitochondria vs. rest and work in skeletal muscle, *PLoS One*, 2015, **10**, e0117145.
- 51 J. Mishra and A. Kumar, Improvement of mitochondrial NAD⁺/FAD⁺-linked state-3 respiration by caffeine attenuates quinolinic acid induced motor impairment in rats: implications in Huntington's disease, *Pharmacol. Rep.*, 2014, **66**, 1148–1155.
- 52 K. Kluckova, L. F. Dong, M. Bajzikova, J. Rohlena and J. Neuzil, *Methods in Molecular Biology*, Humana Press, New York, 2015, pp. 181–194.
- 53 M. J. Hosseini, F. Shaki, M. Ghazi-Khansari and J. Pourahmad, Toxicity of copper on isolated liver mitochondria: impairment at complexes I, II, and IV leads to increased ROS production, *Cell Biochem. Biophys.*, 2014, **70**, 367–381.
- 54 T. Tanaka, K. Nagashima, N. Inagaki, H. Kioka, S. Takashima, H. Fukuoka, H. Noji, A. Kakizuka and H. Imamura, Glucose-stimulated single pancreatic islets sustain increased cytosolic ATP levels during initial Ca²⁺ influx and subsequent Ca²⁺ oscillations, *J. Biol. Chem.*, 2014, **289**, 2205–2216.
- 55 Y. Zhu, H. Xu and K. Huang, Mitochondrial permeability transition and cytochrome c release induced by selenite, *J. Inorg. Biochem.*, 2002, **90**, 43–50.