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Duality of effect of La^{3+} on mitochondrial permeability transition pore depending on the concentration

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Abstract In order to explore the role of mitochondria in proliferation promotion and/or apoptosis induction of lanthanum, the mutual influences between La^{3+} and Ca^{2+} on mitochondrial permeability transition pore (PTP) opening were investigated with isolated mitochondria from rat liver. The experimental results revealed that La^{3+} influence the state of mitochondria in a concentration-dependent biphasic manner. La^{3+} in nanomolar concentrations, acting as a Ca^{2+} analog, entered mitochondrial matrix via the RuR sensitive Ca^{2+} channel and elevated ROS level, leading to opening of PTP indicated by mitochondrial swelling, reduction of $\Delta\Psi_m$ and cytochrome *c* release. Inhibition of PTP with 10 μM CsA attenuated the effects of La^{3+}. However, micromolar concentrations La^{3+} acted mainly as a Ca^{2+} antagonist, inhibiting PTP opening induced by Ca^{2+}. We postulated that this action of La^{3+} on mitochondria through interaction with Ca^{2+} might be involved in the proliferation-promoting and apoptosis induction by La^{3+}.

Keywords Lanthanum ion · Mitochondria · Calcium ion · Permeability transition pore

Abbreviations

Ln	Lanthanides
Rh123	Rhodamine 123
$DCFH_2$-DA	2′7′-Dichlorofluorescein diacetate
CCCP	Carbonyl cyanide *m*-chlorophenylhydrazone
CsA	Cyclosporin A
RuR	Ruthenium red
TES	2-[[Tris (hydroxymethyl) methyl]amino]-1-ethanesulfonicacid
EGTA	Ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
cyt-*c*	Cytochrome *c*
PTP	Permeability transition pore
MCU	Mitochondrial calcium uniporter
$\Delta\psi_m$	Mitochondrial membrane potential
ROS	Reactive oxygen species

Introduction

Lanthanides (Ln) have been known for their diverse biological effects (Wang et al. 1999). The most

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noteworthy one is that they promote both proliferation and apoptosis of cell. The correlation between these two outcomes is not clear (Greisberg et al. 2001). It is generally accepted that the events occurring in mitochondria play crucial role in regulation of life or death of cells. The mitochondrial events in the early stage of apoptosis include opening of permeability transition pore (PTP) of mitochondrial membrane, irreversible dissipation of membrane potential ($\Delta\psi_m$), and the release of cytochrome *c* (cyt-*c*). PTP is a multiprotein complex whose molecular composition and its regulation is not well understood. The PTP opening was proposed to regulate by factors including Ca^{2+}, ADP, and ROS (Anna et al. 2004) and possibly linked with regulation of $\Delta\psi_m$ (Liu et al. 2006).

Previously, we found that La^{3+}, one of lanthanide ions, promotes proliferation of NIH 3T3 via the extracellular La^{3+} induced ERK signaling pathway (Yu et al. 2006). But how does the action turned to be pro-apoptotic is still unknown. Since the across membrane transport of lanthanides was possible, the direct action on mitochondria in association with proliferation/apoptosis related events cannot be excluded, particularly for mitochondria-mediated apoptosis. It is now widely accepted that Ca^{2+} is a critical factor for PTP opening. Since Ln^{3+} exhibited structural and chemical similarities to that of Ca^{2+} (Miller and Tormey 1993; Switzer 1978), it is possible that Ln^{3+} may directly affect mitochondria and stimulate mitochondria-mediate apoptosis either as Ca^{2+} analog or intervene the action of Ca^{2+}. It was already known that La^{3+}-induced apoptosis of normal human liver cell line 7,701 was related to changes of mitochondrial status by La^{3+} (Liu et al. 2003).

To investigate the role of mitochondria in the actions of La^{3+} in the apoptotic process, the interaction of La^{3+} with isolated mitochondria and the intervention to the action of Ca^{2+} were studied in the present work. The experimental results revealed that La^{3+} affected the state of mitochondria in a biphasic manner along with change of the concentration. La^{3+} at nanomolar concentrations could induce mitochondrial swelling and irreversible PTP opening. But La^{3+} at micromolar concentrations inhibit Ca^{2+}-induced PTP opening by acting as a Ca^{2+} antagonist.

Materials and methods

Materials

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), cyclosporin A (CsA), rhodamine 123 (Rh123), ruthenium red (RuR), and 2-[[tris(hydroxymethyl) methyl]amino]-1-ethanesulfonicacid (TES), were from Sigma (St. Louis, MO). 2′,7′-dichlorofluorescein diacetate ($DCFH_2$-DA) was from Molecular Probes (Eugene, OR). Mouse anti-horse cyt-*c* monoclonal antibody was from Santa Cruz. La_2O_3 was from Sinopharm Chemical Reagent Beijing Co., Ltd. All other reagents were of analytical grade.

Lanthanide oxides were used to prepare solution of lanthanide chlorides as described previously (Liu et al. 2003). The solutions of inhibitors and other agents were prepared following the direction of suppliers in their good solvent (i.e., water, ethanol, or dimethyl sulfoxide). To run the control experiments, corresponding solvents were added at concentrations not exceeding 0.1%.

S.D. rats (170–200 g) were obtained from Animal Center of Peking University Health Science Center and operated in accordance with the guidelines for the Care and Use of Laboratory Animals.

Isolation and purification of rat liver mitochondria

Rat liver mitochondria were isolated with gradient centrifugation following published procedure (Marzo et al. 1998). In brief, rats were executed decapitation. Livers were rapidly removed, finely chopped and then homogenized with a Dounce-type homogenizer in an ice-cold homogenization buffer (containing: 300 mM sucrose, 5 mM TES, 0.2 mM EGTA, and pH 7.2). The homogenate was then centrifuged for 10 min at 760*g*. The supernatant was collected and centrifuged further for 10 min at 8,200*g*. The pellet was suspended in homogenizing buffer and then added cautiously onto the top of the 60/30/18% Percoll gradient and then centrifuged for 10 min at 8,740*g* again. The lower interface between 60 and 30% Percoll buffer was collected and then suspended in 10× homogenization buffer and centrifuged for 10 min at 6,800*g*. Mitochondria were collected as the final pellet. All of the procedures were operated at 4°C. Protein concentration was determined using the

Bradford method with bovine serum albumin as standard (Bradford 1976). Mitochondria used in the following studies were within 4 h after isolation.

Osmotic swelling assay of mitochondria

Mitochondrial swelling was assessed spectrophotometrically by monitoring the absorbance of mitochondrial suspension at 540 nm (A_{540}) (Ligeret et al. 2004a). Mitochondria suspension (0.5 mg protein/ml) in 1 ml of respiration buffer (250 mM sucrose, 2 mM $MgCl_2$, 20 mM HEPES with 8 mM sodium succinate, pH 7.5) were incubated with La^{3+} in the absence or presence of Ca^{2+}. Kinetic measurements were performed at 30°C in a microplate spectrophotometer (Sunrise, Tecan, Switzerland). The positive controls were run by the same procedure, but RuR, the specific inhibitor of mitochondrial calcium uniporter (MCU), or CsA, the specific inhibitor of PTP was added. Data were analyzed using Microcal Origin 7.5.

Assessment of mitochondrial membrane potential ($\Delta\psi_m$)

Mitochondrial membrane potential was estimated by measuring the fluorescence of Rhodamine 123 labeled mitochondria (Ligeret et al. 2004b). The fluorescence intensity was monitored using a spectrophotometer (RF-5301) (505 nm excitation and 534 nm emission). Briefly, mitochondria suspension (0.5 mg protein/ml) was added to 3 ml of respiration buffer containing 30 nM Rh123. After $\Delta\psi_m$ was established in about 3 min, La^{3+} or other agents were then added to the medium and the fluorescence was monitored for 10 min. The uncoupling agent CCCP (3 μM) was used to run the positive control of $\Delta\psi_m$ dissipation.

Determination of mitochondrial reactive oxygen species

Formation of intramitochondrial ROS was measured with H_2O_2 sensitive probe $DCFH_2$-DA (Luo and Shi 2005). Mitochondria (0.5 mg protein/ml) were suspended in 3 ml of respiration buffer containing 20 μM $DCFH_2$-DA and then La^{3+} was added to the medium. DCF fluorescence of mitochondria suspensions was scanned immediately at 30°C for 20 min on a fluorescence spectrophotometer operating at excitation and emission wavelengths of 488 and 525 nm, respectively.

Detection of cyt-*c* release by western blot analysis

Mitochondria (0.5 mg protein/ml) suspended in respiration buffer were incubated for 20 min at 30°C with La^{3+}. The reaction mixtures were then centrifuged at 13,000*g* for 10 min at 4°C. The supernatant fractions were obtained and centrifuged further at 100,000*g* for 15 min at 4°C to remove mitochondrial membrane fragments. Aliquots of 30 μl of the supernatants were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (15% gel) and then transferred to polyvinyl filters. The membranes were blocked with 5% defatted milk and incubated overnight at 4°C with mouse anti-horse cyt-*c* monoclonal antibody. Blots were visualized with horseradish peroxidase-coupled secondary antibodies and by enhanced chemiluminescence reaction (Morin et al. 2006).

Measurement of mitochondrial Ca^{2+} release and La^{3+} uptake

Mitochondria (0.5 mg protein/ml) were incubated for 30 min at 30°C in 1 ml of respiration buffer with La^{3+} additions. The reaction mixtures were then centrifuged at 13,000*g* for 10 min at 4°C. The calcium content in supernatants released from mitochondria was determined using atomic absorption spectrometer (Perkin-Elmer, Zeemam-5100) (Salvi et al. 2004). The level of lanthanum in mitochondria were determined using inductively coupled plasma-mass spectrometer (ICP-MS, PE-Sciex DRC II).

Statistical analyses

Experiments were repeated at least three times on mitochondria from three different preparations. Every datum was presented as mean ± SD of three or four independent tests. Statistical comparisons were performed using *t* test, and $P < 0.05$ was considered to be of significance.

Results

Effects of La^{3+} on mitochondrial swelling and mitochondrial membrane potential

Mitochondrial swelling was measured as the decrease of A_{540} (Beavis et al. 1985; Ligeret et al. 2004a, b). Shown in Fig. 1, after addition of La^{3+}, the mitochondrial swelling was observed within 55 min. No change in mitochondrial volume was observed upon incubation with very low concentrations (<1 nM) of La^{3+}, but permanent swelling was induced by La^{3+} at 10–100 nM after 30 min. The swelling could be inhibited by 10 μM CsA or 1 μM RuR. However, La^{3+} at concentrations higher than 1 μM did not cause mitochondrial swelling.

The change of $\Delta\psi_m$ was indicated by the change of the fluorescence of the dye Rh123. In energized mitochondria, Rh123 accumulates in the mitochondrial matrix and is subjected to fluorescence quenching. Upon depolarization of $\Delta\psi_m$, Rh123 is released into the surrounding medium, causing an increase in fluorescence intensity (Emaus et al. 1986). The curves given in Fig. 2a indicated the effects of La^{3+}, a rapid collapse of $\Delta\psi_m$ was induced along with mitochondrial swelling (Fig. 1).

As shown in Fig. 2, a rapid decrease of $\Delta\psi_m$ (indicated by sharp increase of fluorescence) was observed within 2 min upon addition of 100 μM La^{3+}. In the range of 1–100 μM, the effect of La^{3+} reduced with decreasing concentration. The half effect concentration was estimated to be ~65 μM. CsA and RuR could only partially inhibit $\Delta\psi_m$ dissipation (shown in Fig. 2c).

Differing from this result, 100 nM of La^{3+} caused breakdown of $\Delta\psi_m$ after ~5 min of delay. Although 10 nM of La^{3+} still resulted in $\Delta\psi_m$ breakdown, but the lag time lengthened to ~10 min. CsA could completely inhibit the decrease of $\Delta\psi_m$ induced by nanomolar concentration of La^{3+}.

In consistent with the results by Salvi et al. (2004), 100 μM Ca^{2+} caused rapid mitochondrial swelling and $\Delta\psi_m$ dissipation (Fig. 3 control). Interestingly, La^{3+} at the concentrations over 10 μM suppressed the swelling and $\Delta\psi_m$ dissipation caused by Ca^{2+}; but La^{3+} at nM concentrations turned to aggravate the effects of Ca^{2+}. As shown in Fig. 3a, b, the addition of 100 μM La^{3+} stopped mitochondrial swelling immediately and attenuated $\Delta\psi_m$ reduction to a level close to that induced by La^{3+} alone. Addition of La^{3+} for 0.5 min later could gradually recover the $\Delta\psi_m$ to the level, which was close to the result of CsA (Fig. 3b).

The effect of La^{3+} on H_2O_2 generation in mitochondria

The influence of La^{3+} on H_2O_2 production in mitochondria was shown in Fig. 4. La^{3+} at 10–100 nM concentrations slightly elevated H_2O_2 levels (by ~40% up), but at 1–100 μM, La^{3+} suppressed H_2O_2 production in a concentration dependent manner.

Measurement of mitochondrial Ca^{2+} release and La^{3+} uptake

The results given in Fig. 5a showed calcium released from mitochondria to the medium in presence of varied concentration of La^{3+}. Comparing with control, La^{3+} below 1 μM did not cause significant Ca^{2+} release from mitochondria, but significantly promoted Ca^{2+} release above ~10 μM. We presumed that this effect might be related to La^{3+} entry into mitochondria. The results in Fig. 5b indicated the uptake of La by mitochondria and it is concentration-dependent. In addition, it is noted that in the presence of RuR (shown in Fig. 5c), entry of nanomolar La^{3+} was significantly inhibited,

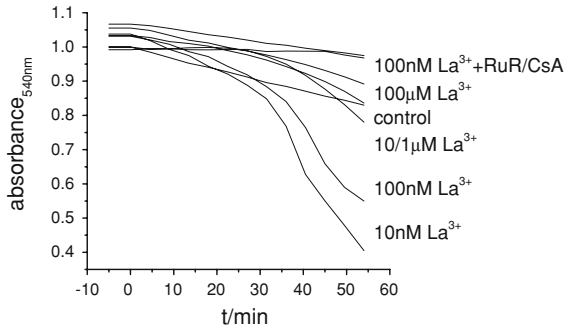


Fig. 1 Effects of La^{3+} on mitochondrial swelling monitored by 540 nm absorbance. Mitochondria (0.5 mg protein/ml) were suspended in Ca^{2+}-free sucrose buffer (250 mM sucrose, 2 mM $MgCl_2 \cdot 6H_2O$, 20 mM HEPES, pH 7.5 containing 8 mM sodium succinate added freshly) and incubated at 30°C with indicated concentrations of La^{3+}

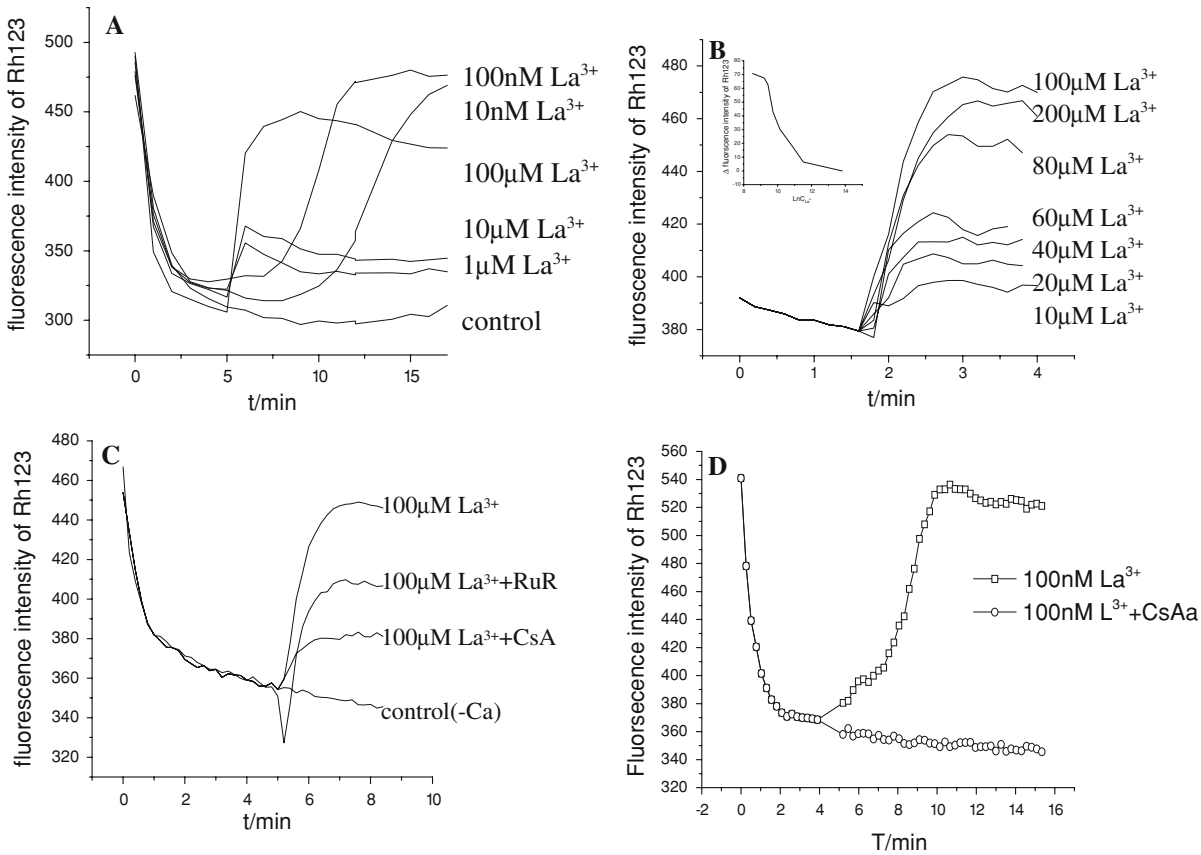


Fig. 2 Effects of La^{3+} on mitochondrial membrane potential ($\Delta\psi_m$) dissipation. **a** Temporal change of $\Delta\psi_m$ upon addition of La^{3+} at the 5th min; **b** rapid change of $\Delta\psi_m$ upon addition of La^{3+} at the 1.5th min. *Inset* the plot of maximal change of $\Delta\psi_m$ versus La^{3+} concentration; **c** effects of CsA and RuR on change of $\Delta\psi_m$ induced by 100 μM of La^{3+}; **d** effects of CsA on change of $\Delta\psi_m$ induced by 100 nM of La^{3+}. Mitochondria were added to respiration buffer (250 mM sucrose, 2 mM $MgCl_2{\cdot}6H_2O$, 20 mM HEPES with 8 mM sodium succinate, pH 7.5) at 30°C supplemented with the potentiometric dye Rh123 and immediately monitored on a fluorescence spectrophotometer (ex/em = 488/525 nm). In preliminary experiments, nonspecific signals and/or false-positive results due to interference of the tested metal ions with $Rh123^+$ were excluded

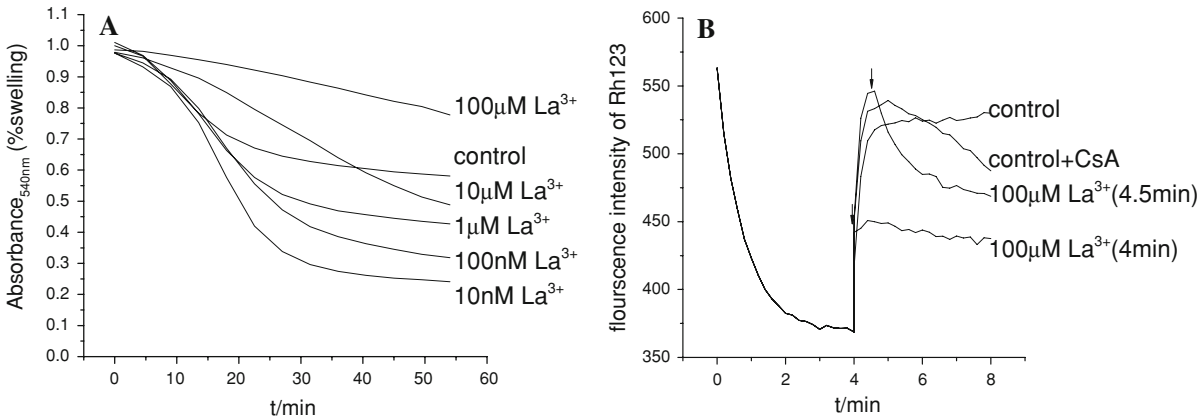


Fig. 3 **a** Influence of La^{3+} on mitochondrial swelling induced by 100 μM of Ca^{2+}; **b** effects of La^{3+} on mitochondrial membrane potential dissipation induced by 100 μM of Ca^{2+}. La^{3+} was added either at the same time with Ca^{2+} (+0 min) or 0.5 min later (+0.5 min). Unless specified, the experiments were carried out as described in Figs. 1 and 2

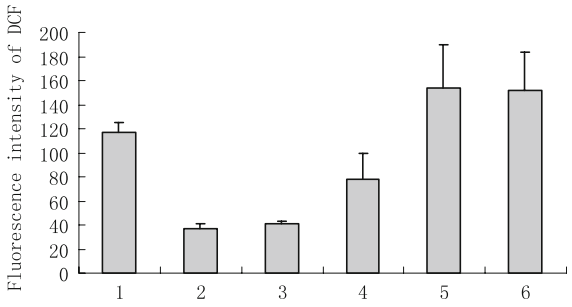


Fig. 4 The level of H_2O_2/total ROS of mitochondria upon incubation with La^{3+}. *1*, Control; *2*, 100 μM La^{3+}; *3*, 10 μM La^{3+}; *4*, 1 μM La^{3+}; *5*, 100 nM La^{3+}; *6*, 10 nM La^{3+}. Mitochondrial was suspended in respiration buffer containing a membrane permeable fluorescent dye $DCFH_2$-DA and the fluorescence at 525 nm were measured after incubation with indicated concentrations of La^{3+} for 10 min

suggesting that La^{3+} at nanomolar concentration might enter mitochondria at least partially through the MCU transporter.

Release of cyt-*c* from mitochondria induced by La^{3+}

PTP opening resulted in release of the apoptotic factor cyt-*c* into the cytosol (Lim et al. 2002). To confirm the induction effect of La^{3+} on PTP, cyt-*c* release from isolated mitochondria upon La^{3+} incubation was analyzed using Western-blot to examine the effect of La^{3+} on cyt-*c* release (Fig. 6). The results showed that La^{3+} under 1 μM cause significant release of cyt-*c*, which could be inhibited by CsA; while La^{3+} above 1 μM did not cause significant cyt-*c* release compared with the control.

Discussion

Previously, we proposed that mitochondria are involved in the mechanism of Ln^{3+}-induced cell apoptosis and is associated with Ln^{3+}-induced ROS generation (Liu et al. 2003). Since the calcium ion

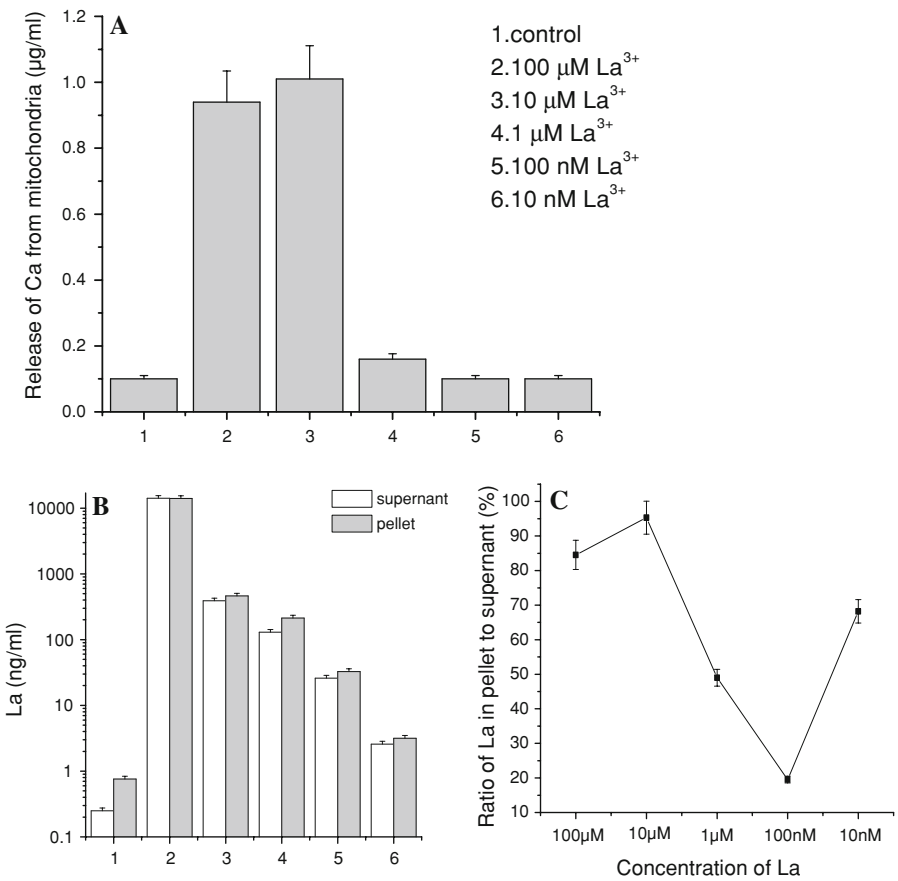


Fig. 5 **a** Release of Ca^{2+} from mitochondria upon incubation with La^{3+}; **b** mitochondrial La^{3+} content upon incubation with La^{3+}; **c** effects of RuR on mitochondrial La^{3+} content for experiments in **b**. *1*, Control; *2*, 100 μM La^{3+}; *3*, 10 μM La^{3+}; *4*, 1 μM La^{3+}; *5*, 100 nM La^{3+}; *6*, 10 nM La^{3+}. Mitochondria (0.5 mg protein/ml) were incubated with various concentrations of La^{3+} in absence or presence of 1 μM RuR for 30 min at 30°C. The reaction mixtures were then centrifuged at 13,000*g* for 10 min at 4°C to obtain supernatant and pellet. The amounts of Ca^{2+} in supernatant were measured using AAS. The amount of La^{3+} in mitochondrial pellet was determined using ICP-MS method

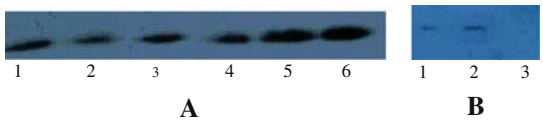


Fig. 6 Western-blot detection of cyt-*c* release from isolated mitochondria upon incubation with La^{3+}. **a** Cyt-*c* release induced by La^{3+} at different concentration. *1*, Control; *2*, control + CsA; *3*, 100 μM La^{3+}; *4*, 10 μM La^{3+}; *5*, 1 μM La^{3+}; *6*, 100 nM La^{3+}. **b** Cyt-*c* release induced by La^{3+} was inhibited by CsA. *1*, Control; *2*, 100 nM La^{3+}; *3*, 100 nM La^{3+}+CsA. Mitochondria (0.5 mg protein/ml) were incubated with various concentrations of La^{3+} in respiration buffer at 30°C for 15 min. After centrifugation at 13,000*g* for 10 min, the supernatant samples were collected and applied to electrophoresis and the amounts of cyt-*c* were analyzed by Western blotting

concentration in the extra-mitochondrial space is a critical determinant on PTP opening and functions, we hereby studied the mutual impact between La^{3+} and Ca^{2+}. It is noted that La^{3+} exhibited concentration-dependent dual effects on mitochondria as following:

La^{3+} at nanomolar concentrations result in opening of mitochondrial permeability transition pore (PTP)

For nanomolar concentrations (10, 100 nM) of La^{3+}, a fraction of La^{3+} bind to and permeated into mitochondria. As shown in Fig. 5b, the amount of mitochondria-bound La^{3+} increased significantly upon incubation with 10 or 100 nM of La^{3+}. RuR could inhibit most of the amounts, suggesting that La^{3+} could at least partly permeate into mitochondria matrix through MCU. In the swelling experiment, both RuR and CsA abolished mitochondrial swelling induced by 100 nM La^{3+} (Fig. 1a), thus the swelling might be due to PTP opening induced by matrix La^{3+}. In Fig. 2a, 100 nM (or 10 nM) of La^{3+} resulted in a time-dependent process of disruption of mitochondrial inner membrane potential ($\Delta\psi_m$), CsA could prevent La^{3+}-induced disruption of $\Delta\psi_m$ (Fig. 2d), suggesting PTP opening was likely related to the process of $\Delta\psi_m$ decrease. Furthermore, release of cyt-*c* was observed (Fig. 6) upon incubation of mitochondria with 100 nM of La^{3+}. Again, CsA could preclude cyt-*c* release, due to La^{3+}-induced PTP opening.

As shown in Fig. 3, nanomolar concentrations of La^{3+} aggravated mitochondrial swelling induced by 100 μM Ca^{2+}. This result presumed that La^{3+}, possibly due to its analogy to Ca^{2+}, induce PTP opening by interacting with PTP proteins in a similar mode to overloaded Ca^{2+} as described (Broekemeier et al. 1989; Rizzuto and Pozzan 2006). Nevertheless, further works on the interaction of La^{3+} with PTP proteins would be appropriate. However, compared with the actions of Ca^{2+} in mitochondrial swelling and $\Delta\psi_m$ reduction (Fig. 3), La^{3+} proceeded much slower, which could be partially due to nM concentration—a kinetic reason.

La^{3+} induced mitochondrial oxidative stress could be an additional factor for the slow process of La^{3+} induced PTP opening. It was seen in Fig. 4 that H_2O_2 level was elevated upon incubation with nanomolar concentrations of La^{3+}; ROS has been shown to induce PTP opening by oxidation of membrane lipid or protein thiol groups (Kowaltowski et al. 2001).

It had been reported that La^{3+} can enter cells via a multipleway mechanism and 80–250 nM of intracellular concentrations of La^{3+} could be reached upon incubation of human keratinocytes cells with lanthanide in μM to mM level (Pillai and Bikle 1992). Therefore, it is reasonable that upon incubation of cells with La^{3+}, nM of La^{3+} will be accumulated in cytoplasm and cause an irreversible opening of PTP in combination with elevated ROS level induced by La^{3+}, thus resulting in cyt-*c* release and finally cell apoptosis.

La^{3+} at micromolar concentrations acts as a Ca^{2+} antagonist

Differently from nanomolar La^{3+}, La^{3+} over 10 μM did not induce mitochondrial swelling (Fig. 1). Disruption of $\Delta\psi_m$ and release of cyt-*c*, an indicator of PTP opening, were not observed (Fig. 6). Although La^{3+} caused a rapid decrease of $\Delta\psi_m$ upon addition of La^{3+} to mitochondria, the $\Delta\psi_m$ could be recovered slowly. CsA or RuR could partially inhibit La^{3+}(μM)-induced decrease of $\Delta\psi_m$ (Fig. 2), suggesting that entry of La^{3+} into mitochondria only partially account for the decrease of $\Delta\psi_m$. Since significant amounts of La^{3+} were observed to bind to mitochondria and RuR did not reduce the amount of La^{3+} with significance (Fig. 5), it might be possible that the rapid dissipation of $\Delta\psi_m$ may be attributed to binding of La^{3+} to mitochondrial inner membrane and also the perturbation of cation influx by entry of La^{3+} into mitochondria through MCU and/or via other mechanisms so far unknown.

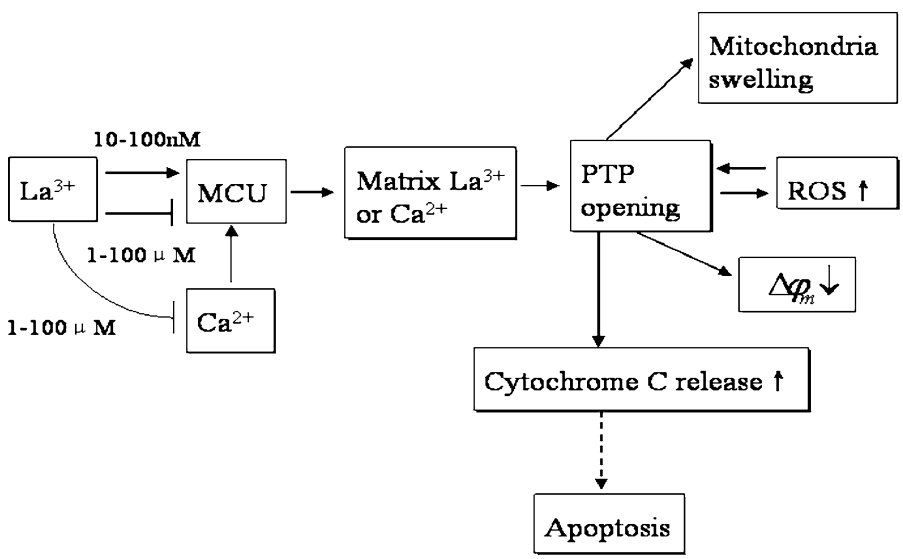
It is not surprising that 100 μM of La^{3+} suppressed mitochondrial swelling and dissipation of $\Delta\psi_m$ induced by Ca^{2+} (Fig. 3) since high concentrations of La^{3+} has been well known as a Ca^{2+} channels blocker (Bakowski et al. 2001). Three possible mechanisms for the action of La^{3+} might lie on:

1. La^{3+} blocked MCU and thus inhibited the entry of Ca^{2+} into mitochondria, which was revealed by release of calcium from mitochondria (Fig. 5). This Ca^{2+} efflux might be the results of blockage of Ca^{2+} channels by La^{3+} as well as displacement of Ca^{2+} upon La^{3+} entry/binding.
2. La^{3+} bound to PTP and blocked the channel. The mitochondrial PTP was known to construct at the inner-/outer-membrane contact sites as a dynamic multi-protein ensemble consisting of voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT), cyclophilin D (Cyp D) and other membrane proteins (Marzo et al. 1998; Nicolli et al. 1996; Petronilli et al. 1993). VDAC has divalent metal binding sites and can be activated by Ca^{2+} (Gincel et al. 2001; Zoratti and Szabo 1995). It is possible La^{3+} bound to metal sites in VDAC and block the channel. Further works would be appropriate to identify the binding site for La^{3+}.
3. High concentrations (>10 μM) of La^{3+} significantly reduced mitochondrial H_2O_2 levels (Fig. 4). Decrease of ROS could be due to the free radical scavenging activity of mM concentration of La^{3+} (Wu et al. 1994) as well as decrease matrix Ca^{2+} level shown in (Fig. 5). It is recognized that mitochondria is one of the Ca^{2+} reservoir (Mcstay et al. 2002) and ROS was observed to elevate with Ca^{2+} concentration in mitochondrial matrix (Carriedo et al. 2000; Starkov et al. 2002).

Overall, La^{3+} over 10 μM might cause blockage of Ca^{2+} and PTP channels, thus resulting in a rapid but recoverable decrease of $\Delta\psi_m$. Analysis of the concentration dependency for this effect gave an apparent half effective concentration (ED_{50}) of ~65 μM. Therefore, it is less possible for La^{3+} to act in this manner inside cells but similar effects should be expected for La^{3+} outside cells.

Conclusion

In summary, the experimental results revealed that La^{3+} influence the state of mitochondria in a biphasic concentration-dependent manner. La^{3+} at nanomolar concentrations could enter mitochondrial matrix via the RuR sensitive Ca^{2+} channel, i.e., MCU, and resulting in ROS elevation, mitochondrial swelling and irreversible PTP opening. This action of La^{3+} is expected to occur in cell and associated with La^{3+}-induced cell apoptotic process. But La^{3+} at micromolar concentrations acts mainly as a Ca^{2+} antagonist, whose effects are expected to occur outside



Scheme 1 Proposed mechanisms of actions of La^{3+} on mitochondrial PTP opening. Nanomolars of La^{3+} enter mitochondrial matrix by Ca^{2+} uniportor and induce PTP opening by binding to PTP proteins as Ca^{2+} analogy as well as increasing ROS levels; while La^{3+} over 10 μM might cause blockage of Ca^{2+} and PTP channels

cells. In overall, actions of La^{3+} are apparently related to its interaction with Ca^{2+} and a postulation of the mechanisms is illustrated in Scheme 1. Further works, e.g., interaction of La^{3+} with PTP proteins, were suggested. The present results should provide new insight in the mechanism by which La^{3+} induce cell apoptosis and be helpful to understand the duality of the biological effects of lanthanides.

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