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Mitochondrial dysfunction induced by different concentrations of gadolinium ion

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HIGHLIGHTS

- Studies on the effect of Gd^{3+} on isolated rat liver mitochondria.
- High concentrations of Gd^{3+} trigger mtPTP opening.
- Chelation of Gd^{3+} with both thiol groups and anionic lipids.

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ABSTRACT

Gadolinium-based compounds are the most widely used paramagnetic contrast agents in magnetic resonance imaging on the world. But the tricationic gadolinium ion (Gd^{3+}) could induce cell apoptosis probably because of its effects on mitochondria. Until now, the mechanism about how Gd^{3+} interacts with mitochondria is not well elucidated. In this work, mitochondrial swelling, collapsed transmembrane potential and decreased membrane fluidity were observed to be important factors for mitochondrial permeability transition pore (mtPTP) opening induced by Gd^{3+}. The protection effect of CsA (Cyclosporin A) could confirm high concentration of Gd^{3+} (500 μM) would trigger mtPTP opening. Moreover, mitochondrial outer membrane breakdown and volume expansion observed clearly by transmission electron microscopy and the release of Cyt *c* (Cytochrome *c*) could explain the mtPTP opening from another aspect. In addition, MBM^+ (monobromobimane$^+$) and DTT (dithiothreitol) could protect thiol (–SH) groups from oxidation so that the toxicity of Gd^{3+} might be resulted from the chelation of –SH of membrane proteins by free Gd^{3+}. Gd^{3+} could inhibit the initiation of mitochondrial membrane lipid peroxidation, so it might interact with anionic lipids too. These findings will highly contribute to the safe applications of Gd-based agents.

1. Introduction

Gadolinium (Gd)-based agents are widely used in magnetic resonance imaging for its efficient spin–lattice relaxation mechanism (Caravan, 2009). For its clinical use, it must chelate with some appropriate ligands because of the high toxicity of Gd^{3+}. Unfortunately, Fe^{3+}, Zn^{2+}, and Cu^{2+} had the ability to displace the gadolinium, resulting in the release of free Gd^{3+} which was then deposited in various tissues (Abraham et al., 2008). Gadolinium accumulated in the environment along with the applications of Gd-based agents, which would eventually lead to an increasing concentration in human, animals and soil (Vahdani et al., 2013). In the vicinity of large cities, river and lake water commonly showed distribution patterns with distinct positive gadolinium anomalies since the first report about gadolinium anomaly in 1996 (Bau and Dulski, 1996; Hennebrüder et al., 2004). In animal studies, intrathecal administration of Gd-based contrast agents in the rat brain caused severe behavioral changes (Toney et al., 2001). Gd-based contrast agents have also been suggested to cause Nephrogenic Systemic Fibrosis especially for patients with renal failure (Broome, 2008).

Gd^{3+}, with biophysical properties similar to Ca^{2+}, permits itself to displace Ca^{2+}. Therefore, it is thought that the biochemical effects induced by Gd^{3+} are probably through interference with intracellular calcium-dependent processes and the route of calcium entry into cells (Adding et al., 2001). However, the specific effects of Gd^{3+} on Central Nervous System are not yet totally clear. Ye and colleagues reported that Gd^{3+} induced the increase of cellular reactive oxygen species and the occurrence of cell death might be

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caused by mitochondrial dysfunction (Ye et al., 2010). Mitochondrial dysfunction is related to mtPTP opening which may be an important factor of the pathogenesis of necrotic cell death (Halestrap and Brenner, 2003). mtPTP is a high-conductance channel located between mitochondrial inner and outer membrane, and formed by adenine nucleotide translocase, voltage-dependent anion channel and cyclophilin D (Vaseva et al., 2012). mtPTP opening could induce mitochondrial biochemical and morphological abnormalities such as the rupture of outer membrane and the release of intermembrane components, which could finally result in cell apoptosis (Green and Kroemer, 2004).

In this background, we attempted to study the effect of Gd^{3+} with different concentrations on mitochondria especially for mtPTP using isolated rat liver mitochondria as the model. As the characteristic signals of mtPTP opening, mitochondrial swelling was monitored by the UV–vis absorption spectroscopy, while the changes of mitochondrial transmembrane potential ($\Delta\psi$) and mitochondrial membrane fluidity were detected by fluorescence spectroscopy. Mitochondrial ultrastructure changes were observed in detail by transmission electron microscopy (TEM).

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), rhodamine 123 (Rh123), hematoporphyrin (HP), Cyclosporin A (CsA), dithiothreitol (DTT), adenosine diphosphate (ADP), rotenone, oligomycin, ethylene glycol tetraacetic acid (EGTA) and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma (St. Louis, MO). All other reagents were of analytical reagent grade, and all solutions were prepared with aseptic double-distilled water.

2.2. Isolation of rat liver mitochondria

Rat liver mitochondria from male Wistar rats (about 250 g) were prepared by standard differential centrifugation according to conventional methods (Belyaeva and Korotkov, 2003). The liver tissue was briefly homogenized in medium A, containing 250 mM sucrose, 2 mM HEPES, 0.1 mM EDTA and 0.1% fatty acid-free BSA (pH 7.4). The protein concentration of mitochondria was determined by the biuret method (Gornall et al., 1949) calibrated with BSA.

2.3. Mitochondrial swelling

Mitochondrial swelling was estimated spectrophotometrically by monitoring the absorbance at 540 nm over 500 s at room temperature (Zhang et al., 2013). Mitochondria (0.5 mg mL^{-1}) were suspended in medium B, containing 200 mM sucrose, 1 mM Tris–MOPS, 1 μM EGTA–Tris, 5 mM succinate, 2 μM rotenone and 3 μg mL^{-1} oligomycin (pH 7.4) and incubated with different concentrations of Gd^{3+}. Medium B mixed with Ca^{2+} (5 μM) before injecting Gd^{3+}. The data were recorded with a UNICO 4802 double-beam spectrophotometer.

2.4. Mitochondrial inner membrane permeabilization

Mitochondrial inner membrane permeability to H^+ and K^+ were measured spectrophotometrically by monitoring the absorbance at 540 nm over 500 s at room temperature. Mitochondria (0.5 mg mL^{-1}) were suspended in K-acetate and K-nitrate mediums respectively to detect mitochondrial inner membrane permeability to H^+ and K^+ (Fernandes et al., 2006). The K-acetate medium contained 135 mM potassium acetate, 5 mM HEPES, 0.2 mM EDTA, 0.1 mM EGTA, 2 μM rotenone and 1 μg mL^{-1} valinomycin (pH 7.1), while the K-nitrate medium contained 135 mM potassium nitrate, 5 mM HEPES, 0.2 mM EDTA, 0.1 mM EGTA, and 2 μM rotenone (pH 7.1).

2.5. Mitochondrial transmembrane potential ($\Delta\psi$)

The change of $\Delta\psi$ was monitored by fluorescence quenching of Rh123 (100 nM), a lipophilic cationic dye (Pacelli et al., 2011). The $\Delta\psi$ was assessed by the LS-55 fluorophotometer (Perkin–Elmer, Norwalk, USA) at λ_{ex} = 488 nm, λ_{em} = 525 nm. Suspended mitochondria (0.5 mg mL^{-1}) in medium B (2 mL) were labeled by Rh123 for 10 min and then Gd^{3+} was added. Finally the fluorescence intensity was recorded after 10 min.

2.6. Mitochondrial membrane fluidity

Dynamic changes of the mitochondrial membrane were analyzed through the changes of fluorescence anisotropy of HP (a membrane lipid bound dye) (Ricchelli et al., 2003). The HP solution (prepared in absolute ethanol) was added into stirred mitochondrial suspension (0.5 mg mL^{-1}). The value of anisotropy (r) could be obtained by measurement of I_Π and $I_\perp$ (i.e. the fluorescence intensities polarized parallel and perpendicular respectively, to the vertical polarization plane of the excitation beam) at λ_{em} = 626 nm (λ_{ex} = 520 nm) about 10 min. The anisotropy (r) is defined by the following equation:

$$r = (I_\Pi - GI_\perp)/(I + 2GI_\perp)$$

where $G = I_\perp/I_\Pi$ is the correction factor for instrumental artifacts.

2.7. Mitochondrial membrane lipid peroxidation

The extent of membrane lipid peroxidation was evaluated by the consumption of oxygen using a Clark-type electrode with magnetic stirring at 25 °C. Mitochondria (1 mg mL^{-1}) were injected into lipid peroxidation medium (1 mL), containing 175 mM KCl, 10 mM Tris–HCl and 3 μM rotenone (pH 7.4) (Fernandes et al., 2006). The medium could alter mitochondrial respiration induced by endogenous substrates. Iron solution was freshly prepared before use and protected from light and oxygen. Membrane lipid peroxidation was initiated by adding 1 mM ADP/0.1 mM Fe^{2+} (Li et al., 2011).

2.8. Mitochondria ultrastructure

Mitochondria under various experimental conditions were fixed for 30 min at 4 °C using glutaraldehyde at a final concentration of 2.5% in 0.1 M cacodilate buffer, then postfixed with 1% osmium tetroxide and dehydrated (Petronilli et al., 2009). The ultrastructure of mitochondria was observed with a JEM-100CX transmission electron microscope.

2.9. Cyt c release

Mitochondria were suspended in medium B and incubated with different concentrations of Gd^{3+} at 30 °C for 1 h (Park and Kim, 2005). The suspensions were centrifuged for 6 min at 13 100 g. The supernatants were analyzed for Cyt *c* using a two-step sandwich ELISA (enzyme-linked immunoabsorbent assay) method (Liu et al., 2003) by a Cyt *c* ELISA kit (Rat Cytochrome *c* ELISA Kit, Novateinbio) in accordance with the manufacturer's instructions. The absorption at 450 nm was recorded on a microtiter plate reader (TECAN).