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Microcalorimetric studies on the energy release of isolated rat mitochondria under different concentrations of gadolinium (III)

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HIGHLIGHTS

- The effect of different concentrations of Gd^{3+} on isolated mitochondrial energy metabolism was studied by microcalorimetry.
- The different changes of low and high concentrations of Gd^{3+} correlate with time.
- High concentration of Gd^{3+} induced the disturbed of energy metabolism.
- Low concentration of Gd^{3+} may promote mitochondrial adaption to physiological stresses.

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ABSTRACT

Gadolinium-based compounds are most widely utilized for paramagnetic contrast agents, but, the toxicological mechanism of gadolinium (Gd) had not been fully elucidated since the first report about Gd anomaly. In this work, we analyzed the effect of Gd^{3+} on mitochondria *in vitro* by microcalorimetry. Microcalorimetry can provide detailed kinetic and thermodynamic information from thermogenic curve. At the tested concentration, Gd^{3+} induced the increase of growth rate constant (k_1). At high concentration (100–500 μM), the maximum power output time (t_m), the decline rate constant ($-k_2$) and the time of activity recovery phase (t_R) decreased with the addition of Gd^{3+} and the maximum power output (P_m) increased. At low concentration (0–100 μM), the changes were different from high concentration. From the results we concluded that the effect of different concentrations of Gd^{3+} had a relationship with time, high concentration of Gd^{3+} induced mitochondrial energy metabolism disturb however low concentration may promote mitochondrial adaption to physiological stresses. The effect of low concentration of Gd^{3+} need more work to elucidate the mechanism. The results of total heat output (Q) and mitochondrial respiratory activities suggested high concentrations of Gd^{3+} could accelerate adenosine triphosphate (ATP) consumption under respiratory system damaged.

1. Introduction

Gadolinium (Gd), a number of rare-earth lanthanides metal, has been widely used in magnetic resonance imaging for its efficient spin-lattice relaxation (Bellin and Van Der Molen, 2008; Caravan, 2009). Great concerns over Gd-based compounds' innocuousness have been raised since the first report about Gd 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 could cause behavioral changes (Toney et al., 2001). Fe^{3+}, Zn^{2+}, and Cu^{2+} had the ability to displace Gd which chelated with some appropriate ligands and Gd is toxic as free ion in environment (Abraham et al., 2008; Adding et al., 2001). The transmetallation and its accumulation in tissues are related to its toxicity closely (Broome, 2008). It has been reported that free Gadolinium ion (Gd^{3+}) could induce cell apoptosis and inhibited neuron mitochondrial metabolic activity at the cellular level (Feng et al., 2010; Ye et al., 2010). Our previously work demonstrated that Gd^{3+} could induce mitochondrial permeability transition pore (mtPTP) opening and mitochondrial outer membrane breakdown (Zhao et al., 2014).

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Mitochondria have long been known as the central importance organelle for adenosine triphosphate (ATP) production in most eukaryotic cells (Hoitzing et al., 2015). And, it has been believed that the outer mitochondrial membrane is constitutively permeable to metabolites involved in oxidative phosphorylation. Microcalorimetric method is a simple and straight-forward method for the measurement of biological process heat flow (Braissant et al., 2010). Modern isothermal microcalorimeters have the advantage of high sensitivity, high accuracy and automaticity which could provide detailed kinetic and thermodynamic information both on simple material and complex biological system (Fan et al., 2014; Han et al., 2012). These advantages make it possible for the study of mitochondria (Li et al., 2010; Xia et al., 2013). And some studies made clear that isolated mitochondria can sustain energy metabolism and perform some metabolic processes with release of heat under adaptive conditions (Dai et al., 2008; Zhao et al., 2011a). A series of thermodynamic and kinetic information can be draw from microcalorimetric curves including some details not observed by other techniques (Chen et al., 2007).

Based on these observations, the present study aims to clarify the influence of Gd^{3+} on isolated rat mitochondria from the aspect of energy metabolism. In the present study, the thermogenic curve of isolated rat mitochondria and the effect of Gd^{3+} on it were studied by the means of microcalorimetry. We obtained the thermokinetic parameters drawn from microcalorimetric curves. It is evident that the loss of cytochrome *c*, an essential player in the respiratory chain, can inhibit the electron transfer process and thus lower ATP levels (Goldstein et al., 2000; Newmeyer and Ferguson-Miller, 2003). Additionally, it was recognised that respiration and ATP synthesis were coupled (Hoitzing et al., 2015). So, we analyze the influence of Gd^{3+} on mitochondrial respiratory.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), HEPES, sucrose, EGTA and EDTA were purchased from Sigma (St. Louis, MO), sodium pyruvate was obtained from Sinopharm Chemical Reagent Co. Ltd., (PR China). 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) (Zhang et al., 2011; Yang et al., 2016). The purified mitochondrial sediment was dispersed in buffer B for calorimetric measurements. Buffer B contained 250 mM sucrose, 0.3 M mannitol, 0.1 mM EDTA. All the operations were performed aseptically at 0–4 °C.

2.3. Protein assay

The protein concentration of mitochondria was determined by the biuret method (Gornall et al., 1949) calibrated with BSA.

2.4. Microcalorimetry determination of isolated rate mitochondria

The metabolic thermogenic curves of isolated mitochondria were performed by using TAM air isothermal calorimeter with ampoule method (Shen et al., 2013). In the experiment, each sealed ampoule contained 2 mL of sample (5 mg protein mL^{-1} energized mitochondria suspension with different concentrations of Gd^{3+}) and 23 mL of air, which provided sufficient O_2 for the mitochondria metabolism (Liu et al., 2000). The ampoules with sample were put into the microcalorimetric system and the temperature was controlled at 30 °C. Meanwhile, a computer was used to record the power-time curves about the heat output of mitochondria continuously. In this experiment, we use pyruvate as energy source and buffer B to supply the osmotic pressure (Xia et al., 2013).

2.5. Mitochondrial respiratory activities influenced by the addition of Gd^{3+}

Mitochondrial respiratory rate was assessed by the consumption of oxygen using a Clark-type electrode (Oxygraph, Hatchtech, Dorchester, UK). Mitochondria (1 mg protein mL^{-1}) were added into a closed glass chamber equipped with magnetic stirring, at 25 °C. The chamber was filled by respiration buffer (1 mL), containing 250 mM sucrose, 20 mM KCl, 10 mM HEPES, 5 mM K_2HPO_4, 2 mM $MgCl_2$ and 1 mM rotenone, pH 7.4 (Puntel et al., 2010). Mitochondrial different respiratory rates were initiated by adding 5 mM succinate and 100 μM ADP (state 3) and 5 mM succinate and 30 μM DNP (uncoupled respiration state) (Yang et al., 2016).

3. Results

3.1. Metabolic thermogenic curves of isolated mitochondria

The heat flux curve of isolated mitochondria energized by pyruvate revealed mitochondrial metabolism (Zhao et al., 2011a). The thermogenic curve can be divided into four phases: lag phase, activity recovery phase, stationary increase phase and decline phase (Fig. 1A). Mitochondria had a slow process of recovery to adapt a new physiological environment in the first 500 min. An obviously activity recovery phase suggested that the isolated mitochondria had a well degree of metabolic activity. After the short activity recovery, mitochondria had a stationary increase phase and lasted about 1000 min. Then mitochondria had a decline phase when the environment nutrition exhausted. Fig. 1B displayed the corresponding metabolic curves of isolated mitochondria with different concentrations of Gd^{3+} and the shape was similar to the control. However, lag phase had a little affect with the addition of Gd^{3+} as shown in Fig. 1B. Moreover, activity recovery phase and stationary increase phase were difficult to separate with the concentration of Gd^{3+} increasing.

3.2. Thermokinetic parameters of mitochondria

To illuminate the effect of Gd^{3+} on mitochondria, the heat production processes were analyzed by thermokinetics (Zheng et al., 2006). As shown in Fig 1B, if the heat output power is P_0 at time = 0, and P_t at time *t*, then thermokinetic equation is:

$$\begin{aligned} P_t &= P_0 \exp(kt) \\ \text{or} \quad \ln Pt &= \ln P_0 + kt \end{aligned} \tag{1}$$

The growth rate constant (k_1) can be obtained from the activity recovery phase as above described Equation (1), as the same to decline rate constant (k_2). And, other parameters were obtained from Fig. 1B directly, which included the maximum power output (P_m), the maximum power output time (t_m), total heat output (Q) and the time of activity recovery phase (t_R) as shown in Table 1.