Effect of Magnesium Sulfate on Mitochondrial Oxygen Consumption Rate

In vitro Study

Amna Al Zaabi and Aiman Al-Rahmani*
Department of Pediatrics, Tawam Hospital, Al Ain, United Arab Emirates

Abstract

Hypoxic insults initiate a cascade of biochemical events that result in irreversible neuronal damage. Magnesium sulfate agent has a possible neuroprotective effect as it can work at different stages of hypoxic brain injury.

Objectives: Magnesium sulfate is used in the neonatal management of HIE as an adjunct medication to reduce Hypoxic Neuronal injury. In this Vitro study, we aimed to examine the efficacy of using magnesium sulfate in human cells to reduce oxygen consumption. We used in-vitro method utilizing foreskin as human cell surrogate.

Method: Foreskin specimens from healthy newborns were collected immediately after circumcision and processed within 1 h for measuring the cellular rate of O2 consumption. Samples were placed in the oxygen phosphorescence analyzer and allowed to run for approximately 1 h. Injection then added magnesium sulfate at different doses into the vial. We analyze the O2 consumption in the samples at different levels of Magnesium Sulfate; the results were plotted using Kaleida Graph TM software.

Results: The rate of respiration reduced with increasing the dose of MgSO4. The Cumulative analysis of cellular respiration rate was before and after an addition of MgSO4. Collectively yielding a (k) value of 0.08 μM O2/min μM O2 min-1 mg- and 0.04 μM O2/min μM O2 min-1 mg- respectively with a significant P-value of <0.001.

Conclusion: Magnesium sulfate reduces the rate of O2 Consumption in a dose-dependent manner.

Keywords: O2 Consumption; Cellular respiration; Bioenergetics; Metabolism; Mitochondrial; Magnesium sulfate; Perinatal asphyxia; Neonates

Abbreviations: Pd phosphor: Pd (II) complex of meso-tetra-(4-sulfonatophenyl)-tetra benzoporphyrin; PBS: Phosphate-buffered saline; 1/r: Phosphorescence Decay Rate; kq: The Second-order O2 Quenching Rate Constant in sec-1 μM-1; k: Rate of Cellular Respiration (μM O2 min-1); k: Rate of Cellular Respiration Corrected by Specimen Weight (μM O2 min-1 mg-1); MgSO4: Magnesium Sulfate.

Background

Brain Injury in Neonates can commonly be the result of perinatal hypoxic-ischemic encephalopathy (HIE), which may lead to significant morbidity or death in the newborn period. The injury to the brain occurs as a result of a combination of systemic hypoxemia; which refers to a compromised arterial oxygen concentration. In addition to a diminished cerebral [1] perfusion that leads to ischemia or insufficient blood flow to the cells to maintain their normal function. The pathogenesis of HIE is an integral sequence of cerebral insults that occur initially with hypoxemia, ischemia and next by oxygenation and reperfusion of the ischemic tissue. Perinatal hypoxia is multi-system organ damage with significant impairments in clotting, renal, and cardiac functions. Magnesium Neuroprotection properties are the results of blocking N-methyl-D-aspartate (NMDA) receptor-mediated calcium entry into the cell. Suppress the release of a neurotransmitter that activated by hypoxia [2] and modulating the actions of pro-inflammatory cytokines and oxygen free radicals.

Antenatal magnesium sulfate is one of the known strategies to reduce perinatal cerebral injury and act as Neuroprotection via several pathways [3,4]. Postnatal MgSO4 treatment of severe perinatal asphyxia patients was shown in several studies to improve neurological outcomes at discharge for term neonates compared to control patients [5]. Meta-analysis of high quality randomizes studies demonstrate that the risk of cerebral palsy is reduced to almost one-third in an infant who received antenatal magnesium sulfate [6].

Furthermore, providing Magnesium sulfate treatment was demonstrated to alter fetal cerebellar gene expression in response to hypoxia that known to contribute to cell death and neurogenesis [7]. These changes negatively affected the newborns for that some studies failed to prove the Neuroprotective effect of MgSO4, which may be due to the dual reaction to the MgSO4 treatment in observations reported for affected newborns [8]. The goal was to conduct in vitro study to investigate the effect of magnesium sulfate on metabolic rate and oxygen consumption in human cells using foreskin as a surrogate biomarker for metabolic rate [9-12].

Methods

Reagents and solutions

Pd (II) complex of meso-tetra-(4-sulfonatophenyl)-tetra benzoporphyrin (Pd phosphor) obtained from Porphyrin Products (Logan, UT). Minimum Essential Medium (MEM Alpha Modification) was purchased from Gibco (labeled as MEM). Phosphate-buffered saline (PBS), glucose oxidase (powder from Aspergillus niger), D (+) glucose anhydrous and remaining reagents were purchased from Sigma-Tau Pharmaceuticals (Gaithersburg, MD, USA).

*Corresponding author: Aiman Al-Rahmani, Department of Pediatrics, Tawam Hospital, Al Ain, United Arab Emirates, Tel: +97137677435; E-mail: arahamani@seha.ae

Received March 20, 2018; Accepted April 13, 2018; Published April 20, 2018


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The Pd phosphor solution (2.5 mg/mL = 2 mM) was made in dH2O and stored at -20°C in small aliquots. The pH was adjusted to ~7.0 with 12 N HCl and stored at -20°C. PBS with and without 5 mM glucose stored at 4°C. Cellular respiration measured in 1 mL sealed vials containing PBS (with and without 5 mM glucose), 3 μM Pd phosphor, and 0.5% fat-free albumin.

**Foreskin specimens**

Sample collection from participants was approved by the Al Ain Medical District Human Research Ethics Committee (Protocol No. 11/59, approved on 16 April 2012; "The phosphorescence oxygen analyzer as a screening tool for metabolic disorders"). Informed consent obtained from each patient.

**Oxygen instrument**

The rate of cellular respiration was determined using phosphorescence analyzer that measures the concentration of dissolved O2 as a function of time [13,14]. The Pd phosphor had a maximum absorption at 625 nm and a maximum phosphorescence emission at 800 nm.

The samples exposed to light flashes (10 per sec) from a pulsed light-emitting diode array that peaked at 625 nm. Emitted phosphorescent light was filtered at 800 nm and detected by Hamamatsu photomultiplier tube. Amplified phosphorescence was digitized at 1-2 MHz using an analogue/digital converter (PCI-DAS 4020/12 I/O Board) with 1 to 20 MHz outputs. Pulses captured at 1.0 MHz [13].

The phosphorescence decay rate characterized by a single exponential; \( I = \text{Set}/\tau \), where \( I \) = Pd phosphor phosphorescence intensity. The values of \( 1/\tau \) were linear with dissolved O2: \( k_q[O_2] \), where \( 1/\tau = \text{the phosphorescence decay rate in the presence of O}_2 \), \( 1/\tau^0 = \text{the phosphorescence decay rate in the absence of O}_2 \), and \( k_q = \text{the second-order O2 quenching rate constant in sec}^{-1} \mu\text{M}^{-1} \) [14].

Cellular respiration measured in 1 mL vials sealed from air. The vials contained PBS (with and without 5 mM glucose), 3 μM Pd phosphor, and 0.5% fat-free albumin. The temperature was controlled by a circulating water bath (Precision ± 0.5°C). O2 concentration, calculated using the equation: \( k_q[O_2] \) [14], decreased linearly with time, indicating the kinetics of mitochondrial O2 consumption was zero-order. The rate of respiration (\( k \), in μM O2/min) was, thus, the negative of the slope \( d[O_2]/dt \). A program was developed using Microsoft Visual Basic 6, Microsoft Access Database 2007, and Universal Library components [13]. Magnesium sulfate injected into the vials at 60 min of respiration at different doses of 10 μg, 5 μg and 2.5 μg (stock conc. 50 mg/ml).

**Statistical analysis**

Data were analyzed using SPSS statistical package (version 19). The nonparametric test (2 independent variables) Mann-Whitney was used to compare treated and untreated sample.

**Results**

Our study demonstrates a dose dependent reduction of cellular Respiratory rate with the addition of Magnesium Sulfate (Figure 1A-C). We compared the slope of cellular respiration at base line and then after adding Magnesium sulfate at increasing dosages (5 and 10 μM respectively).

We demonstrated a significantly reduced slope of cellular respiration with increasing dose of Mg Sulfate (P < 0.001, for both 5 μM and 10 μM in comparison to baseline) (Figure 2).

**Discussion**

Magnesium Sulfate (MgSO4) when given to mother 24 h prior to the delivery of a preterm infant, was reported to reduce mortality in addition to a favorable effect of improving neurological outcome, reduces gross motor dysfunction evident a 2 years evaluation [15].

A major research challenge remains, that is to develop effective methods to limit or prevent perinatal neuronal damage and reduce the severity or prevalence of cerebral palsy and associated co-morbidities following preterm births, and in infants who are exposed to a Hypoxic ischemic insult in the perinatal period [4].

There is increasingly encouraging evidence that MgSO4 is neuroprotective in mature rat pups when given after experimental focal
There are animal studies that evaluated neuronal injury reduction with Sulfate in perinatal Hypoxic ischemic insult was not studied [16].

For neonatal HIE (NRP reference), the added benefits of magnesium. Therapeutic Hypothermia is emerging as a standard of care treatment for Hypoxic ischemic encephalopathy (HIE) in the neonatal period. As combined efficacy of therapeutic hypothermia in the treatment of proven to be more neuroprotective in higher doses of MgSO4.

Citation: ISSN: 1948-593X
J Bioanal Biomed, an open access journal

P value of <0.001.

The mean baseline cellular respiration slope (k) was 0.061 ± 0.017 μM O2 min⁻¹ mg⁻¹. Then cellular respiration slope (k') significantly reduced to rate of 0.046 μM O2 min⁻¹ mg⁻¹ (n = 10, P <0.001).

Figure 1: Figures shows that the cellular Respiratory rate (k') reduction in a dose-dependent manner. The rate of respiration (k') was reduced with increasing the dose of MgSO4.

Figure 2: Cumulative analysis of cellular respiratory rate before and after addition of MgSO4 collectively yielding a (k') value of 0.08 μM O2/min µM O2 min⁻¹ mg⁻¹ and 0.04 μM O2/min µM O2 min⁻¹ mg⁻¹ respectively with a significant P value of <0.001.

The major limitation of this study was the inability unable to run same samples of the foreskin with different drug concentration due to limited cellular viability over a short period. Therefore an alternative approach was followed utilizing one concentration of MgSO4 for each set of sample, then to compare the cellular respiration mean values of each group of samples to the other groups which were treated with different doses.

The results of this novel in vitro study in human cells may indicate that infants who are exposed to treatment with MgSO4 may have similar systemic and cerebral hemodynamics; nevertheless, the Neuroprotection properties of MgSO4 are excreted by lowering the cerebral fractional tissue oxygen extraction cFTOE compared to non-treated controls. These findings also suggest that similar reduction in cerebral metabolism may be a component of the neuroprotective action of antenatal administered MgSO4 [20].

We previously reported a similar experimental method studying neonatal foreskin cellular respiration. Demonstrating that neonatal cellular respiration is highly sensitive to critical temperatures (33°C vs. 35°C) [1].

References


