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Effect of Magnesium Sulfate on Mitochondrial Oxygen Consumption Rate *In vitro* Study

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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 O_2 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 O_2 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 $MgSO_4$. Collectively yielding a (*k*) value of 0.08 μM O_2/min μM O_2 min^{-1} mg- and 0.04 M O_2/min μM O_2 min^{-1} mg- respectively with a significant P-value of <0.001.

Conclusion: Magnesium sulfate reduces the rate of O_2 Consumption in a dose-dependent manner.

Keywords: O_2 Consumption; Cellular respiration; Bioenergetics; Metabolism; Mitochondrial; Magnesium sulfate; Perinatal asphyxia; Neonates

Abbreviations: Pd phosphor: Pd (II) complex of meso-tetra-(4-sulfonatophenyl)-tetrabenzoporphyrin; PBS: Phosphate-buffered saline; 1/τ: Phosphorescence Decay Rate; k_q: The Second-order O_2 Quenching Rate Constant in sec^{-1} μM^{-1}; k: Rate of Cellular Respiration (μM O_2 min^{-1}); k_c: Rate of Cellular Respiration Corrected by Specimen Weight (μM O_2 min^{-1} mg^{-1}); $MgSO_4$: 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 cerebra [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 $MgSO_4$ 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 $MgSO_4$, which may be due to the dual reaction to the $MgSO_4$ 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).

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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 O_2 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 = Set/τ, where I = Pd phosphor phosphorescence intensity. The values of 1/ were linear with dissolved O_2: $k_q[O_2]$, where $1/\tau$ = the phosphorescence decay rate in the presence of O_2, $1/\tau°$ = the phosphorescence decay rate in the absence of O_2, and k_q = the second-order O_2 quenching rate constant in sec^{-1} μM^{-1} [14].

Cellular respiration measured in 1 mL vials sealed from air. The vials contained PBS (with and without 5mM glucose), 3 µM Pd phosphor, and 0.5% fat-free albumin. The temperature was controlled by a circulating water bath (Precision ± 0.5°C). O_2 concentration, calculated using the equation: $k_q[O_2]$ [14], decreased linearly with time, indicating the kinetics of mitochondrial O_2 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).

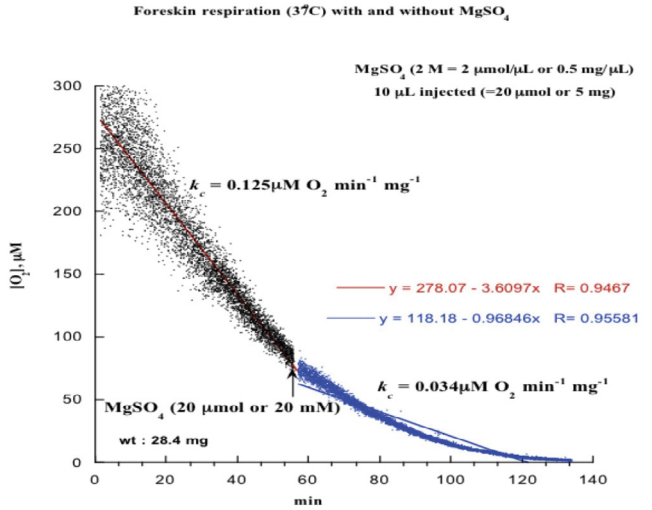
We demonstrated a significantly reduced slope of cellular respiration with increasing dose of Mg Sulfate ($P < 0.001$, for both 5 µM and 10 µM in comparisson to baseline) (Figure 2).

Discussion

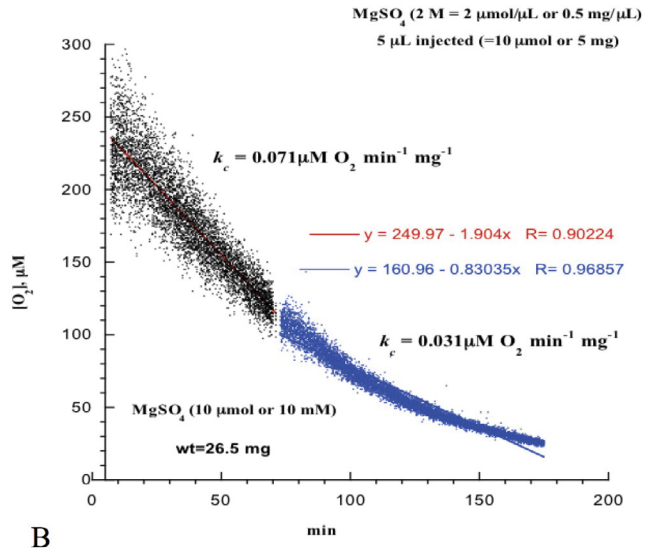
Magnesium Sulfate ($MgSO_4$) when given to mother 24 h prior to the delivery of a preterm infant, was reported to reduce mortality in addition to a favorable effect of improving neurological outcome, reduces gross motor dysfunction evident a 2 years evaluation [15].

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].

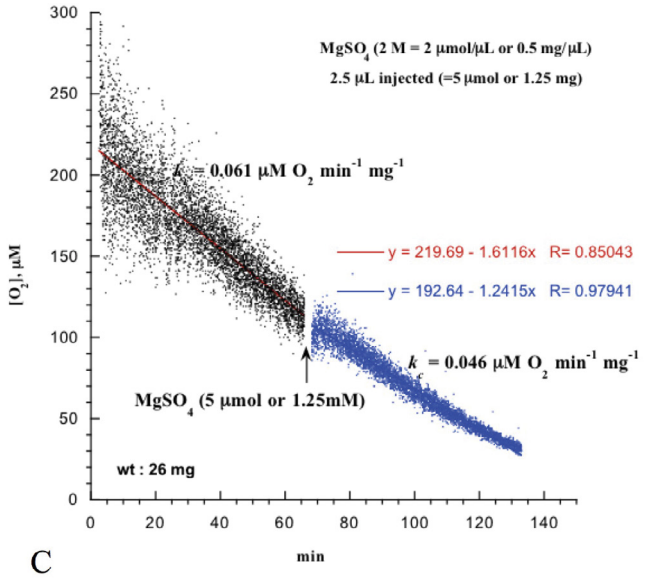
There is increasingly encouraging evidence that $MgSO_4$ is neuroprotective in mature rat pups when given after experimental focal



Shows the rate of cellular respiration (k_c) (µM O_2 min^{-1} mg^{-1} before addition of $MgSO_4$ k_c = 0.125 µM O_2 min^{-1} mg^{-1} . Then after $MgSO_4$ was injected at a dose of 10 µL (= 20 µM or 5 mg), the cellular respiration slope (k_c) was significantly reduced to 0.034 ± 0.008 µM O_2 min^{-1} mg^{-1} (n = 11, P < .001).



Shows the effect of lower dose of $MgSO_4$ at 5 µL (= 10 µM or 2.5 mg) on the rate of cellulrar respiration(k_c) from a mean baseline cellural respiration slope (k_c) of 0.071 ± 0.05 µM O_2 min^{-1} mg^{-1}, to a (k_c) rate of 0.031 ± µM O_2 min^{-1} mg^{-} (n = 10, $p < 0.001$).



Shows the effect of injecting a lower $MgSO_4$ dose at 2.5 µL (=5 µM or 1.25 mg). The mean baseline cellural respiration slope (k_c) was 0.061 ± 0.017 µM O_2 min^{-1} mg^{-} Then cellural respiration slope (k_c) significantly reduced to rate of 0.046 µM O_2 min^{-1} mg^{-} (n = 10, P <0.001).

Figure 1: Figures shows that the cellular Respiratory rate (k_c) reduction in a dose-dependent manner . The rate of respiration (k_c) was reduced with increasing the dose of $MgSO_4$.

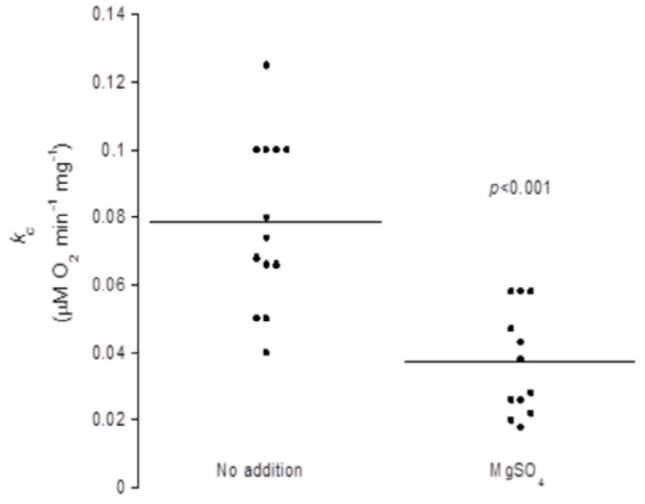


Figure 2: Cumulative analysis of cellular respiratory rate before and after addition of $MgSO_4$ collectively yielding a (k) value of 0.08 µM O_2/min µM O_2 min^{-1} mg^{-} and 0.04 µM O_2/min µM O_2 min^{-1} mg^{-} respectively with a significant P value of <0.001.

stroke lesions with an effect that is dose dependent, and a response proven to be more neuroprotective in higher doses of $MgSO_4$.

There are no published studies in human subjects evaluating the combined efficacy of therapeutic hypothermia in the treatment of Hypoxic ischemic encephalopathy (HIE) in the neonatal period. As Therapeutic Hypothermia is emerging as a standard of care treatment for neonatal HIE (NRP reference), the added benefits of magnesium Sulfate in perinatal Hypoxic ischemic insult was not studied [16]. There are animal studies that evaluated neuronal injury reduction with $MgSO_4$ in adult rats, where pre-treatment with $MgSO_4$ before global cerebral ischemia [17], combined with mild spontaneous hypothermia after ischemia, reduced hippocampal neuronal loss more than $MgSO_4$ or hypothermia alone [18] Thus, combination therapy is promising, but it has not yet been tested in neonatal HIE. One multicenter clinical trial studying the safety and efficacy of hypothermia with $MgSO_4$ (Mag Cool) is currently listed on Clinical Trials Gov. [16].

The results of this study demonstrated a significant (P<0.01) reduction in foreskin cellular respiration (as a cellular surrogate for the neonatal living cells) with an addition of $MgSO_4$. This effect of $MgSO_4$ was dose dependent whereas the concentration of $MgSO_4$ increased in the studied tissue, the cellular respiration (as reflected by oxygen consumption) decreased by 50% from the cellular respiration baseline in tissue samples, which were not treated with $MgSO_4$ [19].

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 $MgSO_4$ 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 $MgSO_4$ may have similar systemic and cerebral hemodynamics; nevertheless, the Neuroprotection properties of $MgSO_4$ 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 $MgSO_4$ [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].

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