Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

C. Tsompos1*, C. Panoulis2, K Toutouzas3, A. Triantafyllou4, C. G Zografos5, K. Tsarea6, M. Karamperi7, A. Papalois8

1ConstantinosTsompos: Department of Gynecology, Anticancer Hospital of Thessaloniki "Theageneio" Thessaloniki, Hellas
2ConstantinosPanoulis: Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Hellas
3Konstantinos Toutouzas: Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Hellas
4AggelikiTriantafyllou: Department of Biologic Chemistry, Athens University, Athens, Attiki, Hellas
5C. G Zografos: Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Hellas
6KalliopiTsarea: Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Hellas
7Maria Karamperi: Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Hellas
8Apostolos Papalois: Experimental, Educational and Research Center ELPEN European University Cyprus, School of Medicine

Abstract

Aim: This study calculated the effects on serum magnesium (Mg) levels, after treatment with either of 2 drugs: the erythropoietin (Epo) and the antioxidant lazaroid (L) drug U-74389G. The calculation was based on the results of 2 preliminary studies, each one of which estimated the certain influence, after the respective drug usage in an induced ischemia reperfusion (IR) animal experiment.

Materials and methods: The 2 main experimental endpoints at which the serum Mg levels were evaluated was the 60th reperfusion min (for the groups A, C and E) and the 120th reperfusion min (for the groups B, D and F). Specially, the groups A and B were processed without drugs, groups C and D after Epo administration; whereas groups E and F after the L administration.

Results: The first preliminary study of Epo presented a non significant hypomagnesiemic effect by 0.19% ± 0.10% (p-value=0.9197). However, the second preliminary study of U-74389G presented a significan hypermagnesiemic effect by 0.36% ± 1.66% (p-value=0.8228). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that L is 1.823808-fold more hypermagnesiemic than Epo (p-value=0.0000).

Conclusion: The anti-oxidant capacities of U-74389G ascribe1.823808-fold more hypermagnesiemic effects than Epo (p-value=0.0000).

Keywords: ischemia; erythropoietin; U-74389G; serum magnesium levels; reperfusion

1. INTRODUCTION

The lazaroid U-74389G (L) may be not famous for its hypermagnesiemic capacity (p-value=0.8228). U-74389G as a novel antioxidant factor, implicates exactly only 261 published studies. The ischemia reperfusion (IR) type of experiments was noted in 19.15% of these studies. A tissue protective feature of U-74389G was obvious in these IR studies. The U-74389G chemically known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant complex, which prevents the lipid peroxidation either iron-dependent, or arachidonic acid-induced one. Animal kidney, liver, brain microvascular endothelial cells monolayers and heart models were protected by U-74389G after IR injury. U-74389G also attenuates the leukocytes; down-
Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

regulates the proinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents antishock property.

Erythropoietin (Epo) even if is not famous for its hypomagnesiemic action (p-value=0.9197), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 31,312 published biomedical studies, only a 3.68% of them negotiate the known type of IR experiments. Nevertheless, Epo as a cytokine, it is worth of being studied about its effects on serum magnesium (Mg) levels too. This experimental work tried to compare the effects of the above drugs on a rat induced IR protocol. They were tested by calculating the serumMg levels alterations.

2. MATERIALS AND METHODS

2.1. ANIMAL PREPARATION

The Vet licenses under 3693/12-11- 2010 & 14/10-1-2012 numbers, the granting company and the experiment location are mentioned in preliminary references. The human animal care of Albino female Wistar rats, the 7 days pre-experimental *ad libitum* diet, the non-stop intra-experimental anesthesiologic techniques, the acidometry, the electrocardiogram, the oxygen supply and post-experimental euthanasia are also described in preliminary references. Rats were 16 – 18 weeks old. They were randomly assigned to six (6) groups consisted in N=10. The stage of 45 min hypoxia was common for all 6 groups. Afterwards, reperfusion of 60 min was followed in group A; reperfusion of 120 min in group B; immediate Epo intravenous (IV) administration and reperfusion of 60 min in group C; immediate Epo IV administration and reperfusion of 120 min in group D; immediate U-74389G IV administration and reperfusion of 60 min in group E; and immediate U-74389G IV administration and reperfusion of 120 min in group F. The dose height assessment for both drugs are described at preliminary studies as 10 mg/Kg body mass.

Ischemia was caused by laparotomic clamping the inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reperfusion. After exclusion of the blood flow, the protocol of IR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through inferior vena cava catheter. The Mg levels were determined at 60th min of reperfusion (for A, C and E groups) and at 120th min of reperfusion (for B, D and F groups). Along, non-relation was raised between Mg values with animals’ mass (p-value=0.9551).

3. STATISTICAL ANALYSIS

Table 1 presents the (%) hypomagnesiemic influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) hypermagnesiemic influence of U-74389G regarding reperfusion time. Chi-square tests were applied using the ratios which produced the (%) results per endpoint. The outcomes of chi-square tests are depicted at Table 3.

Table 1. The (%) hypomagnesiumic influence of erythropoietin in connection with reperfusion time

<table>
<thead>
<tr>
<th>Hypomagnesiemia+SD</th>
<th>Reperfusion time</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1.00% +16.80%</td>
<td>1h</td>
<td>0.8596</td>
</tr>
<tr>
<td>-1.09% +13.06%</td>
<td>1.5h</td>
<td>0.7248</td>
</tr>
<tr>
<td>-3.19% +10.32%</td>
<td>2h</td>
<td>0.3729</td>
</tr>
<tr>
<td>+4.22% +13.14%</td>
<td>reperfusion</td>
<td>0.1794</td>
</tr>
<tr>
<td>-0.19% +0.10%</td>
<td>interaction</td>
<td>0.9197</td>
</tr>
</tbody>
</table>

Table 2. The (%) hypermagnesiumic influence of U-74389G in connection with reperfusion time

<table>
<thead>
<tr>
<th>Hypermagnesiemia+SD</th>
<th>Reperfusion time</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1.33% +11.02%</td>
<td>1h</td>
<td>0.7033</td>
</tr>
<tr>
<td>-0.28% +12.36%</td>
<td>1.5h</td>
<td>0.9171</td>
</tr>
<tr>
<td>-1.90% +16.81%</td>
<td>2h</td>
<td>0.7161</td>
</tr>
<tr>
<td>+4.28% +11.40%</td>
<td>reperfusion</td>
<td>0.1074</td>
</tr>
<tr>
<td>+0.36% +1.66%</td>
<td>interaction</td>
<td>0.8228</td>
</tr>
</tbody>
</table>

Table 3: The U-74389G / erythropoietin magnesium alterations efficacies after chi-square tests application

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>[95% Conf. Interval]</th>
<th>p-values</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.331108</td>
<td>1.324786</td>
<td>1.33746</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.2605466</td>
<td>.2587177</td>
<td>.262384</td>
<td>0.0000</td>
</tr>
</tbody>
</table>
Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

| 0.5961915 | 0.594238 | 0.5981254 | 0.0000 | 2h |
| 1.013227 | 1.010824 | 1.015635 | 0.0000 | reperfusion |
| 1.823808 | 1.806514 | 1.841268 | 0.0000 | interaction |

4. RESULTS

The successive application of chi-square tests revealed that U-74389G caused hypermagnesiemia by 1.331108-fold [1.324786 - 1.33746] more than Epo at 1h (p-value=0.00007), by 0.2605466-fold [0.2587177 - 0.2623884] less hypomagnesiemiathan Epo at 1.5h (p-value=0.0000), by 0.5961915-fold [0.594238 - 0.5981254] less hypomagnesiemiathan Epo at 2h (p-value=0.0000), by 1.013227-fold [1.010824 - 1.015635] more hypermagnesiemiap-value=0.0000) without drugs andby 1.823808-fold [1.806514 - 1.841268] more hypermagnesiemiathan Epo whether all variables have been considered (p-value=0.0000).

5. DISCUSSION

The unique available study investigating the hypermagnesiemic effect of U-74389G was the preliminary one1. Although the most famous activities of neuroprotection and membrane-stabilization properties, it accumulates in the cell membrane, protecting vascular endothelium from peroxidative damage but hardly penetrates the blood-brain barrier. It elicits a beneficial effect in otoxicity and Duchenne muscular dystrophy. It increases γgt, superoxide dismutase (SOD) and glutathione (GSH) levels in oxygen-exposed cells. It treats septic states and acts as immunosuppressant in flap survival. It prevents the learning impairments, it delays the early synaptic transmission decay during hypoxia improving energetic state of neurons. It shows antiproliferative properties on brain cancer cells and is considered as a new promising anti inflammatory drug for the treatment of reperfusion syndrome in IR injuries.

The same authors confirmed2 the short-term hypomagnesiemic effect of Epo preparations in non iron deficient individuals. Lee MY et al determined3 changes in blood Mg2+ and tMg after hemorrhage identifying mechanism and origin of the changes in blood Mg2+. Hemorrhagic shock produced significant increases in blood Mg2+, but significant decreases in RBC tMg. During hemorrhagic shock, K+, anion gap, and BUN showed significant positive correlations with changes in blood Mg2+ level, while Ca2+, pH, and T-CHO were correlated with Mg2+ in a negative manner. Thus, hemorrhagic shock induced an increase in both blood-free Mg2+ and tMg, resulted from Mg2+ efflux from metabolic damaged cell with acidosis and ATP depletion in rats. Wang AF et al found4 potassium, calcium, magnesium and to some extent, boron and zinc concentrations decreased in both Betula species and additionally manganese in B. pendula likely connected with impaired root functioning, which was not yet fully recovered from GW. Chubanov V et al uncovered5 new cellular functions of the transient receptor potential cation channel subfamily M member 7 (TRPM7) in situ including its crucial role in Ca2+ signaling and Ca2+ dependent cellular processes. Genetic inactivation of this bi-functional protein revealed its crucial role in Ca2+ signalling, Mg2+ metabolism, immune responses, cell motility, proliferation and differentiation. Reilly-O'Donnell B et al suggested6 that dysregulated Zn2+ homeostasis alters the function of both RyR2 and MG23 and that both ion channels play a key role in diastolic SR Ca2+ leakage in H9C2 cells exposed to ischemic conditions. Koning G et al noticed7 that mRNAs/miRNAs is involved in mitochondrial function and metabolism were modulated by MgSO4. Metabolomic analysis (H+-NMR) disclosed that MgSO4 attenuated hypoxia-ischemia (HI)-induced increases in succinate and prevented depletion of high-energy phosphates. MgSO4 pretreatment preserved mitochondrial respiration, reducing ROS production and inflammation after HI. Therefore, we propose that MgSO4 evokes preconditioning via induction of mitochondrial resistance and attenuation of inflammation in seven-day-old rats. Poore LA et al diagnosed8 urolithiasis, with uroliths identified as magnesium calcium phosphate carbonate in composition in a hand-reared 5-month-old steenbok. Groom KM et al used9 of peripartum maternal administration of magnesium sulphate for neuroprotection and corticosteroids for fetal lung maturity, to prevent adverse neonatal outcomes. Wolf A et al included10 the delta opioid receptor agonist D-Ala-Leu-enkephalin (DADLE), the gasotransmitter hydrogen sulfide, combinations of adenosine, lidocaine, and magnesium (ALM) or D-beta-hydroxybutyrate and melatonin (BHB/M) and therapeutic hypothermia in hibernation as a treatment of hemorrhagic shock. Koning G et al resulted11 in a transient serum Mg2+ concentration elevation at 3 h post i.p. injection), reduced brain injury by 74% in grey matter and 64% in white matter after MgSO4 administration i.p. 24 h prior to the induction of HI. MgSO4
provides a marked preconditioning protection both in postnatal day 4 and in postnatal day 5 in mice. Seyama T et al attenuated the loss of premyelinating oligodendrocytes (pre-OL) markers, such as myelin basic protein and Olig2, in ipsilateral pericallosal white matter and decreased the number of CD-68-positive microglia and TUNEL-positive cells in vivo; also inhibited lactate dehydrogenase release from pre-OL induced by oxygen glucose deprivation in vitro after antenatal maternal pretreatment with MgSO₄ in a neonatal rat model. Mu YP et al found that increasing the magnesium concentration attenuates vasoconstriction and improves vasodilatation via release of nitric oxide. Pulmonary hypertension is associated with endothelial dysfunction resulting in the suppression of magnesium modulation of vasodilatation. Magnesium regulates vascular functions by altering the vascular responses to vasodilator and vasoactive agonists and affects endothelial function by modulating endothelium-dependent vasodilatation in hypertension. Removal of extracellular magnesium suppressed vasoreactivity of pulmonary hypertension (PA)s. A high concentration of magnesium (4.8 mm) inhibited ET-1-induced vasoconstriction in endothelium-intact or endothelium-disrupted pulmonary arterial (PA)s of normoxic and chronic hypoxia (CH)-treated mice, and enhanced the ACh-induced production of NO in PAs of normoxic mice. Moreover, magnesium enhanced ACh-induced vasodilatation in PAs of normoxic mice. Hence, in this study we demonstrated that increasing the magnesium concentration can attenuate the ET-1-induced contractile response. Bennet L et al affirmed that MgSO₄ significantly reduced the total duration, number of seizures, seizure amplitude, average seizure burden, the total number of seizures (P < 0.001), peak seizure amplitude (P < 0.05) and seizure burden (P < 0.005) after hypoxia-ischaemia. Finally, seizures showed a circadian profile with peak seizures between 04.00 and 06.00 h on the first and second day post-UCO. Collectively, these results suggest that MgSO₄ may have utility in treating perinatal seizures and has sexually dimorphic effects in preterm fetal sheep. Chollat C et al decreased the induced lesions' sizes and inflammatory cytokine levels, prevented cell death, and improved long-term behavior and reduced risks of cerebral palsy after antenatal magnesium sulfate preconditioning therapy in hypoxia models, including hypoxia-ischemia, inflammation, and excitotoxicity in various species (mice, rats, pigs). Dhillon SK et al gave treatments such as antenatal glucocorticoids and magnesium sulphate to reduce the risk of death and complications after preterm birth in mothers who are at risk of premature delivery and have high rates of diabetes and antepartum infection/inflammation. Samsonia M et al have shown factors such as - enhancement of the antioxidant systems of neurons by acetylcysteine, inhibition of glutamate excitotoxicity by lamotrigine and correction of ion stress by magnesium ions, critically increase the time prior to apnea. Accordingly, the combination of magnesium sulfate, lamotrigine and acetylcysteine is able to protect brain cells from the damaging effects of oxygen-glucose deprivation and can be effectively used for pharmacological correction of hypoxic brain damage in acute ischemic stroke. Meloni BP et al indicated that magnesium is only neuroprotective when combined with post-ischemic hypothermia in cerebral ischemia models. Ogunyemi D et al associated untreated anxiety/depression with magnesium exposure (aOR = 1.82) for independent maternal outcomes in subjects more likely being Non-Hispanic Whites, using tobacco, public insurance and illegal substances, unmarried, and having medical complications of pregnancy. Kumar V et al found calcium, magnesium, and phosphorous amounts decreased but the strongest decline was seen for potassium, whereas, photosynthesis-related parameters were hardly affected, thus accumulated carbohydrates under all stresses and anthocyanins under Hpx exclude carbohydrate limitation in rosettes. Daher I et al noted that MgSO₄ prevented sensorimotor alterations in pups, with the same efficacy in males and females, displayed anti-apoptotic and anti-inflammatory effects without deleterious side effects, tended to prevent these motor and cognitive deficits only in females, while it prevented global brain tissue damage in both sexes. Liu Y et al directly removed the intratumoral oxygen via the oxidation reactions of Mg₂Si nanoparticles and later efficiently block the rapid reoxygenation via tumor blood vessels by the resultant SiO₂ microsheets for cancer starvation therapy. Chollat C et al found the resulting lesion sizes and inflammatory cytokine levels decreased, prevented cell death, and improved long-term cognitive and motor behaviours in models of hypoxia, hypoxia-ischemia, inflammation, and excitotoxicity after magnesium sulphate preconditioning in various animal species. However, antenatal magnesium sulphate as a neuroprotectant showed prevention of CP at 2 years in infants born preterm when administered to females at risk of imminent preterm birth in humans. Plush K et al tended to find reduced the negative impacts of birth hypoxia, higher vitality scores immediately after birth (p <
Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

0.10), quicker to colostrum suck and higher day one blood glucose concentrations in Mg piglets than Cont piglets (p < 0.05) during the peri-natal period. Dumanska H et al observed hypoxia-induced reduction of voltage-dependent magnesium blockade of evoked N-methyl-D-aspartate receptor (NMDA) response in retinocollicular synaptic transmission. Bagheri G et al revealed that MS reduced the number and intensity of necrotic insults. The Bax/Bcl2 ratio and malondialdehyde (MDA) levels were significantly decreased in a dose-dependent manner in the MS-treated rats compared with the positive control group, while a significant dose-dependent increase in Akt expression, a pro-survival protein, was observed. In addition, MS administration reduced pro-apoptotic indice levels, ameliorated histological insults, favorably modulated oxidative status and increased Akt expression levels, indicating a possible neuroprotective effect in the case of CO poisoning resulting in protecting against CO-induced cerebral injury in male Wistar rats. Lisukha LM et al determined the content of magnesium (Mg2+) electrolytes having a multidirectional signification in different age groups in non-stimulated mixed saliva of children, with disorders of autonomous nervous system (ANS), who were born and are permanently residing at radioactive contaminated territories after application of intermittent normo-baric hypoxia (INH) of sanogenic level. Adam GO et al revealed that the levels of magnesium (Mg2+), and calcium (Ca2+) were significantly decreased whereas Ca2+/Mg2+ were significantly increased than those found pre toxemia induction in acute lipopolysaccharide (LPS)-toxemia models. Li T et al showed that MLB prevented the elevation in right ventricular systolic pressure and the increase in ratio of wall thickness to vessel external diameter of pulmonary arteries in PAH rats, attenuated phenotypic transformation of PASMCs (decrease in α-smooth muscle actin while increase in osteopontin), accompanied by downregulation of NADPH oxidase (NOX) (NOX2 and NOX4) protein levels, decrease of ROS and H2O2 production, and suppression of the phosphorylation of ERK. Pradhan T et al significantly related the mortality with the number of seizure episodes and time interval between seizure onset and administration of magnesium sulphate. Early detection of hypertension and management with magnesium sulphate for eclampsia can help to minimize the maternal and fetal adverse outcomes in patients with eclampsia. Lin P et al noticed that magnesium lithospermate B (MLB) can promote renal microcirculation, improves renal function, fibrosis and inflammation and significantly reduces 24hUp, Scr, BUN, SBP and DBP levels. The expression of FN, Col-I, CTGF and IL-6 were down-regulated by MLB treatment whereas significantly increased RBF. MLB reversed the expression of nNOS (neuronal nitric oxide synthase), HIF-1α (hypoxia inducible factor-1) and VEGF in rats with CRF in the remnant kidney with CRF. Xu L et al measured increased proliferation with Mg extracts under hypoxia but not under normoxia. Under normoxia and with Mg extracts, HUVEC migration exhibited a bell-shaped curve. Under normoxia and increased Mg concentrations, a general negative effect was measured on early (migration) and late (tubulogenesis) angiogenesis; as magnesium degradation is an oxygen-dependant process in vitro in human primary endothelial cells. Li T et al showed hypertrophic features (increases in the ratio of RV weight to tibia length, cellular size, and hypertrophic marker expression), accompanied by upregulation in expression of NADPH oxidases (NOX2 and NOX4) and vascular peroxidase 1 (VPO1), increases in hydrogen peroxide (H2O2) and hypochlorous acid (HOCl) production and elevation in phosphorylation levels of ERK; attenuated by treating rats with Magnesium lithospermate B (MLB) in hypoxic pulmonary arterial hypertension (PAH) SD rats. Solevåg AL et al targeted the acute brain injury in neonatal asphyxia phase after allopurinol, melatonin, noble gases such as xenon and argon, and magnesium administration also. Xing J et al reported the transient receptor potential melastatin 7 (TRPM7) as an endogenous magnesium channel to promote the proliferation of SMCs. The TRPM7 currents and intracellular magnesium level in PASMCs were also reduced by pulmonary arterial hypertension (PAH) stimuli. Functionally, TRPM7 inhibition with waixenicin A or knockdown promoted and reversely, its overexpression inhibited the proliferation and apoptosis resistance of PASMCs in Sprague-Dawley rats. Lingam I et al noticed that MgSO4 bolus and infusion doubled serum magnesium (0.72 vs 1.52 mmol/L) with modest (16%) rise in CSF. In Mg+HT compared with HT, there was overall reduced cell death (p = 0.01) and increased oligodendrocytes (p = 0.002) in a piglet model of term neonatal encephalopathy. Wagner S et al generated an integrated picture of the physiological response to progressing hypoxia monitoring cytosolic dynamics of magnesium adenosine 5'-triphosphate, free calcium ion concentration, pH, NAD redox status, and glutathione redox status in parallel, linked to transcriptional and metabolic responses. Mi F et al observed that Mg2+ protected...
Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

HT22 cells against hypoxia-induced damages by upregulating microRNA-221 (miR-221) and NGF in mouse hippocampal HT22 cells. El Faragy MS et al assessed\(^39\) that MgSO\(_4\) may have an added effect for the reduction in brain injury through the expression of S100-B, a marker of brain injury, in neonates infants with HIE who are receiving melatonin. Xu L et al noticed\(^40\) that Mg degradation products increased human umbilical cord perivascular (HUCPV) migration, remarkably increased cytokines (e.g., c-c motif chemokine ligand 2 and vascular endothelial growth factor) and MSC mineralization, decreased and increased the mesenchymal stem cells (MSC) proliferation in transwell and in heterotypic-contact coculture, respectively in transwell under hypoxia.

According to above, table 3 shows that U-74389G has 1.823808-fold [1.806514 - 1.841268] more hypermagnesemic than Epo whether all variables have been considered (p-value=0.0000); a trend attenuated along time, in Epo non-deficient rats. A meta-analysis of these ratios from the same experiment, for 29 other seric variables, provides comparable results (table 4)\(^41,42\).

Table 4. A U-74389G / erythropoietin efficacies ratios meta-analysis on 29 hematologic variables\(^41,42\).

6. CONCLUSION

The anti-oxidant agent U-74389G was proved having 1.823808-fold [1.806514 - 1.841268] more hypermagnesemic effect than Epo whether all variables have been considered (p-value=0.0000); a trend attenuated along the short term time frame of the experiment in rats. A biochemical investigation remains about how U-74389G mediates in these actions.
Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process

REFERENCES


Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process


Comparison of the Magnesium Alterations after Erythropoietin and U-74389G Process


Copyright: © 2019 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.