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CLINICAL STUDIES

Intracellular magnesium in elderly patients with heart failure: Effects of diabetes and renal dysfunction

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Abstract

Hypomagnesemia is frequent in diabetes mellitus (DM), while renal dysfunction (RD) may be associated with hypermagnesemia. Severe cardiac arrhythmias and other adverse clinical manifestations are frequent in heart failure (HF), in DM and in RD. Depletion of intracellular magnesium (icMg), which may coexist with normal serum Mg, might contribute to these deleterious effects. However, icMg content in normomagnesemic HF patients with RD or DM has not been studied. We assessed total icMg in peripheral blood mononuclear cells (PBMC) from 80 normomagnesemic furosemide-treated HF patients who were divided as follows: subgroups A (DM), B (RD), C (DM and RD), and D (free of DM or RD). PBMC from 18 healthy volunteers served as controls. IcMg content (μg/mg cell protein) in HF was lower compared to controls (1.68 ± 0.2 vs. 2.4 ± 0.39, $p < 0.001$). In the entire HF group, a significant inverse correlation was evident between icMg and serum creatinine ($r = -0.37$) and daily furosemide dosages ($r = -0.121$). IcMg in the HF subgroups A, B, C, and D was 1.79 ± 0.23, 1.57 ± 0.23, 1.61 ± 0.25, and 1.79 ± 0.39, respectively ($p = 0.04$ between A and B, $p = 0.08$ between B and D, and non-significant in the remaining comparisons). Serum Mg, potassium, calcium, furosemide dosages and left ventricular ejection fraction were comparable in all subgroups. In conclusion, icMg depletion was demonstrable in PBMC, which may be responsible for some of the adverse clinical manifestations in HF patients. In particular, icMg depletion in RD might contribute to cardiac arrhythmias in this patient group. Mg supplementation to normomagnesemic HF patients might therefore prove beneficial.

Keywords: Heart failure; Diabetes mellitus; Renal failure; Magnesium

Introduction

Despite advances in the diagnosis of heart failure (HF) and its treatment, mortality rates remain high. Within 3-year following diagnosis, 50% of such patients die, 35% of them by sudden death [1,2]. Cardiac arrhythmias in HF may be secondary to impaired

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electrolyte balance, including hypomagnesemia and/or intracellular magnesium (icMg) depletion [2,3].

HF is often associated with both diabetes mellitus (DM) and renal dysfunction (RD). DM is a leading cause of hypomagnesemia in patients with or without HF [4,5]. RD, even mild, is a marker of poor survival in HF [6–8]. RD state is frequently associated with normo- or hypermagnesemia, while cardiac arrhythmias as well as sudden deaths are common both in HF and in RD [2,7,9,10].

Serum Mg levels do not necessarily reflect the state of icMg. Depletion of icMg stores, which may coexist with normal serum Mg values [11,12], might be responsible for various adverse clinical effects, including cardiac arrhythmias and sudden death [3,13,14]. However, icMg content in HF associated with RD or DM has not been studied. The present study was therefore undertaken to assess total icMg content in peripheral blood mononuclear cells (PBMC) from normomagnesemic HF patients in which HF was or was not associated with DM or RD.

Methods

Patients

The study included 80 patients (age range 55–90 years), with systolic or diastolic HF [left ventricular ejection fraction (LVEF) 15–70%], who had been clinically stable for at least one month. HF was chronic, grades II–IV New York Heart Association (NYHA) and had been present for $\geqslant 6$ months. All patients had been on daily furosemide treatment at a dosage $\geqslant 40$ mg for more than 6 months. The diagnosis of chronic HF was based on data from previous hospitalizations and/or outpatient facilities records. These included typical symptoms (shortness of breath, orthopnea, paroxysmal nocturnal dyspnea), physical signs (edema, pulmonary rales, gallop rhythm, displaced left ventricular apical impulse) and radiographic evidence of pulmonary congestion (pulmonary venous redistribution, basal or perihilar vascular blurring, Kerley B lines, pulmonary edema and pleural effusions).

RD and DM were known for at least 6 months. RD was defined as serum creatinine >1.4 mg/dL. Diagnosis of DM was based on medical history, glycosylated hemoglobin (HbA_{1c}) $>6\%$ and persistent fasting hyperglycemia (>126 mg/dL). All DM patients were treated with antihyperglycemic drugs. Data regarding LVEF were obtained from two-dimensional echocardiography performed within 6 months prior to starting the investigation. Only normomagnesemic patients were included in the study. Patients with preexisting conditions for which Mg administration was indicated [abnormally low (<1.9 mg/dL) serum Mg levels] or contraindicated [high (>2.6 mg/dL) serum Mg levels] were excluded. Patients receiving Mg-containing drugs and patients with conditions known to affect Mg metabolism, such as acute renal failure, maintenance dialysis, malignant diseases, thyroid or parathyroid abnormalities, active liver diseases or alcoholism were also excluded.

The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethics committee. All participants provided their written informed consent prior to starting the study.

Study design

HF patients were divided in 4 subgroups as follows: (A) 25 subjects with DM, (B) 17 patients with RD, (C) 30 subjects with both DM and RD, and (D) 8 patients free of DM or RD. In addition, 18 healthy volunteers of comparable age, who underwent regular physical check-ups at least once a year, were recruited from the hospital staff and served as a control group.

Blood procurement and PBMC isolation

In total, 20 mL of heparinized whole blood samples were drawn in the morning for PBMC isolation. In addition, 7 mL of blood were separately procured for serum Mg, calcium, potassium, creatinine, blood hemoglobin (Hb), and HbA_{1c} determinations.

PBMC were isolated by gradient centrifugation on Ficoll-Hypaque (Amersham, UK). The isolated cells were washed in phosphate-buffered saline (PBS, pH 7.4) not containing calcium or Mg salts, resuspended in 1 mL PBS, counted in a hemocytometer and stored at $-70\,^{\circ}C$ until used for total icMg determination.

Total icMg determination

Total intracellular content of Mg in PBMC has been reported by a number of authors to reflect the total status of the ion in various tissues, including cardiomyocytes [14–17]. Since the main goal of the present study was to determine with maximal accuracy and precision the total Mg status of the cells, including cytosol and intracellular stores, rather than to investigate transient transmembrane fluxes of ionized Mg, the atomic absorption spectrometry method was employed in this investigation.

The samples were washed 3 times in PBS, and the cell pellets completely digested by 24 h incubation in 1 mL concentrated HCl. Total Mg determination was performed on atomic absorption spectrophotometer SpectrAA800 (Varian-Tecktron, Australia) by a method

described elsewhere [18,19]. In brief, 300 μL of completely digested PBMC samples were placed in polystyrene test tubes containing 5 mL of matrix diluent. The latter was prepared from 12.5 g lanthanum oxide dissolved in 200 mL of concentrated HCl (stock solution) and diluted to 2500 mL with water distilled by reverse osmosis (working solution). All reagents, including water, were of atomic absorption purity grade. A standard curve was built based on three different concentrations of Mg chloride stock standard solution. The prepared samples were dispersed and burned in a high temperature flame using a mixture of nitric oxide-acetylene gases. Three samples of a known concentration were tested 10 times each to assess intra-assay precision, which yielded a coefficient of variability of 4.5%. For inter-assay precision determination, 20 separate measurements were performed on the same sample, yielding a coefficient of variability of 5.5%. Total protein content of the samples was determined by the Bradford method [20], and the results subsequently calculated as microgram of Mg per milligram of cell protein.

Statistical analysis

Nonparametric Kruskal–Wallis test within analysis of variance (ANOVA) was used to compare the data. Differences between the results yielding p values <0.05 were considered statistically significant. Pearson's correlation coefficient (r) was calculated to evaluate correlations between total icMg content and serum creatinine, Mg, blood HbA_{1c}, LVEF, or furosemide dosages.

Results

Basal clinical and biochemical data of the participants are presented in Table 1. No significant differences between the study subgroups were evident with respect to mean age, LVEF, furosemide dosages, Hb values, serum Mg, potassium, and calcium. In all subgroups, percentages of patients using angiotensin-converting enzyme (ACE) inhibitors and NYHA grading were comparable.

Total icMg in PBMC from the entire group of HF patients was significantly lower compared to the healthy controls (Fig. 1, 1.68 ± 0.2 vs. 2.4 ± 0.39 μg/mg cell protein, $p<0.001$). Within the entire HF group, a statistically significant inverse correlation was found between icMg and serum creatinine ($r=-0.37$). Likewise, inverse correlation between icMg and furosemide dosages was found significant ($r=-0.121$). No significant correlation was found between icMg levels and LVEF, HbA_{1c} or serum Mg.

Fig. 1 illustrates the values of total icMg content in the HF patients and controls. The lowest icMg values were demonstrable in subgroup B (RD, 1.57 ± 0.23 μg/mg cell protein). They were significantly lower compared to controls ($p<0.001$) as well as to subgroup A (DM, 1.79 ± 0.23 μg/mg cell protein, $p=0.04$), and were lower than in subgroup D (free of DM or RD, 1.79 ± 0.39 μg/mg cell protein), in this case, however, the difference did not reach statistical significance ($p=0.08$). No statistically significant difference was observed between subgroups A, C, and D ($p>0.1$ in each comparison).

No correlation was found between icMg content and HbA1c levels in DM, or between serum creatinine values and icMg in the RD subgroups.

Table 1. Baseline characteristics of the patients

Characteristic	Entire HF group ($n=80$)	Subgroup A DM ($n=25$)	Subgroup B RD ($n=17$)	Subgroup C DM + RD ($n=30$)	Subgroup D No DM or RD ($n=8$)
Age (years)	72.2±10.8	68.7±10	77±6	71±15	77±12.3
Male/female	47/33	14/11	13/4	16/14	4/4
LVEF (%)	39.5±14.2	42.1±13	38.7±17.8	38.2±14	35.7±14.5
NYHA grade II/III/IV	50/22/8	16/7/2	11/4/2	18/9/3	5/2/1
Furosemide dosage (mg/day)	60.1±31	55.6±30	54.1±22.1	66.7±38.8	57.8±21
Using ACE inhibitors (%)	53	52	41	57	62
Hemoglobin (g/dL)	12.2±1.5	12.1±1.7	12.4±1.5	11.7±1.6	13±1.2
Serum creatinine (mg/dL)	1.47±0.65*	0.97±0.2	1.77±0.4*	1.98±0.64*	0.9±0.16
Hemoglobin A_{1c} (%)	7.8±1.7**	8.2±1.31**	6.1±0.5	8.3±1.7**	5.8±0.4
Serum magnesium (mg/dL)	2.02±0.26	1.98±0.25	2.1±0.25	1.99±0.26	2.03±0.3
Serum potassium (mmol/L)	4.3±0.6	4.46±0.62	4.34±0.47	4.42±0.9	3.97±0.5
Serum calcium (mg/dL)	9.34±0.5	9.53±0.6	9.46±0.59	9.24±0.5	9.16±0.45

Values are mean±SD unless stated otherwise.
*Significantly higher compared to non-RD subgroups.
**Significantly higher compared to non-DM subgroups.

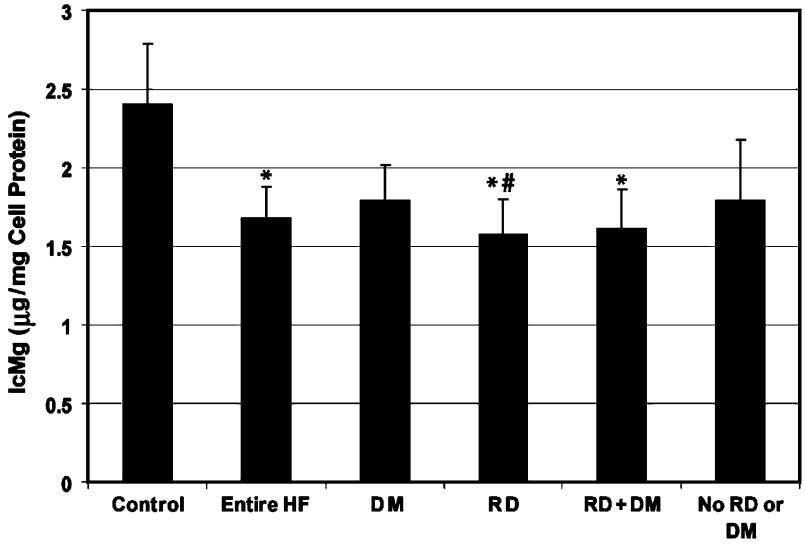

Fig. 1. Total icMg content in the HF patients. Abbreviations: icMg-intracellular magnesium; Control-healthy controls; HF-the entire heart failure group; DM-diabetes mellitus (subgroup A); RD-renal dysfunction (subgroup B); RD + DM (subgroup C); No RD or DM (subgroup D); *-significantly decreased compared to control; ♯-significantly decreased compared to DM.

Discussion

The present study demonstrates significantly decreased total icMg levels in PBMC from HF patients and more so from patients in whom HF was associated with RD. Our data confirms a previously published report showing decreased icMg content in PBMC from normomagnesemic furosemide-treated HF patients compared to healthy controls [21]. Moreover, we found significant inverse correlation between icMg and maintenance furosemide dosages. We, however, have taken a step further, differentiating HF patients into specific subgroups according to coexisting conditions. We have found that while coexistent DM did not affect total icMg in HF patients, the values of icMg were decreased in HF patients with RD, alone or combined with DM.

RD has been shown to be associated with a high incidence of cardiac arrhythmias and cardiac arrests [9,10]. Annual mortality among patients with RD is 10%, up to 38% of the latter are related to sudden arrhythmic events [10]. Possible causes for arrhythmias may include cardiac ischemia, prolongation of QT-interval, various electrolyte disorders, acid base aberrations as well as deposition of calcium or aluminum in heart tissue [10]. With respect to icMg content in RD patients, various, even contradictory results have been reported, possibly due to differences in methodologies or experimental protocols [22–24]. Most of these studies concentrate on patients with severe RD. Patients with mild to moderate RD have not yet been substantially investigated. Moreover, no information has been thus far available concerning icMg in HF associated with RD. Our results demonstrated significantly reduced icMg in patients with mild to moderate RD compared to those with DM or free of DM and RD. Moreover, significant inverse correlation was found between icMg and serum creatinine levels in the entire HF group. Low icMg might be a contributory factor in the higher incidence of arrhythmias in HF patients with coexistent RD. The picture might prove different in patients with advanced and/or end stage RD, calling for additional studies on the issue. It is noteworthy however, that with respect to any marker of HF severity, no significant difference was demonstrable between the RD patients and the distinct HF subgroups. One might conclude that within the present experimental setup patients with RD did not have worse HF than any other subgroup.

Several investigations have indicated that total icMg in circulating PBMC reflects the total body Mg status [15–17]. If so, it seems conceivable that reduced PBMC Mg content in HF patients might reflect Mg depletion in other tissues, including cardiocytes [14,15,21]. If this observation is substantiated in future studies, this could provide an additional therapeutic avenue to a variety of symptoms common in HF and thus far attributed to the HF condition per se. Thus, in addition to cardiac complications, muscular weakness, fatigue, anorexia, and nausea, common to HF patients, might be ameliorated by Mg supplementation [25]. However, in a limited number of publications reporting empirically prescribed Mg supplementation to normomagnesemic HF patients, those with RD were a priori excluded [19,26]. Factors known to contribute to Mg depletion in HF patients do indeed, include reduced dietary intake, along with impaired intestinal absorption and/or exaggerated urinary excretion generated by diuretics

[3,27,28]. Moreover, activation of the neurohormonal and renin–angiotensin–aldosterone systems leads to stimulation of aldosterone and antidiuretic hormone secretion, which inhibits tubular magnesium reabsorption and thus exaggerates urinary Mg loss [3,27,28]. Taken together, this would indicate that regular Mg supplementation might prove beneficial for this patient category.

In addition to cardiac arrhythmias, Mg deficiency in HF patients may generate impairment of myocardial metabolism, further reduction in cardiac contractility, coronary and systemic arterial constriction [3,27,28]. Moreover, Mg depletion is contributory to increased catecholamine secretion [29], the hallmark of HF pathophysiology [3,27,28]. In turn, catecholamines have been demonstrated, in vitro and ex vivo, to regulate icMg loss in lymphocytes from patients with essential arterial hypertension, thus creating a loop wherein Mg deficiency induces catecholamine secretion while elevated catecholamines stimulate further Mg loss [30,31]. Both processes are contributory to vasoconstriction and further elevation of systemic blood pressure. Mg has been demonstrated to inhibit catecholamine release by a mechanism involving blockade of voltage-gated calcium channels, thus breaking the deleterious loop and lowering systemic blood pressure [32]. With this respect, the beneficial effects of Mg supplementation appear to be convincing.

It should be noted that our study has been performed on a relatively small number of patients, especially concerning the subgroup free of DM and RD. In addition, in a study performed on humans, only PBMC were available for icMg content assessment. We could only suggest that our results might be extrapolated on cardiocytes or other tissues [14–17]. The mentioned limitations of the present study call for future investigations.

In conclusion, depletion of icMg stores was demonstrable in PBMC from HF patients, especially with concomitant RD, which may be responsible for some of the adverse clinical manifestations in these patients. Measurement of total icMg in PBMC by atomic absorption spectrometry is a relatively simple and not too costly procedure. Mg supplementation monitored by total icMg, instead of serum Mg, assessment may prove advantageous for normomagnesemic HF patients.

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