The relationship between concentrations of magnesium and oxidized low density lipoprotein and the activity of platelet activating factor acetylhydrolase in the serum of patients with type 1 diabetes

Małgorzata Wegner¹, Aleksandra Araszkiewicz², Dorota Zozulińska-Ziółkiewicz², Bogna Wierusz-Wysocka², Anna Pioruńska-Mikołajczak¹, Maria Pioruńska-Stolzmann¹

¹ Department of General Chemistry, Chair of Chemistry and Clinical Biochemistry, Poznan University of Medical Sciences, Poznan; ² Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznan, Poland

Correspondence: M. Wegner, Department of General Chemistry, Grunwaldzka St.6, 60-708 Poznan, Poland <malgoweg@ump.edu.pl>

Abstract. The study was aimed at comparing the concentration of metabolic parameters, the serum concentration of oxidized low density lipoproteins (oxLDL) and the activity of platelet activating factor acetylhydrolase (PAF-AH) in the relation to the serum concentration of magnesium (Mg) in patients with type 1 diabetes (DM1). DM1 patients (n=78) were divided into 2 groups: patients with low serum Mg concentration (<0.7 mmol/L, group 1, n=34) and patients with reference levels of Mg (≥0.7 mmol/L, group 2, n=44). A control group (n=24) of healthy subjects was also recruited. Our results showed that DM1 patients had lower serum Mg concentrations than the control group. It was found that parameters of poor metabolic control and lipid profile are not related to the serum Mg concentration in DM1 patients. However, both the Mg concentration and the PAF-AH activity are independently related to the serum oxLDL concentration. In group 1 the oxLDL concentration and the PAF-AH activity were higher than in group 2, and the control group. Two groups of DM1 patients did not show any differences with regard to the metabolic control. Therefore, the oxidative modification of LDL and the higher activity of PAF-AH are related with the low Mg status; however, no relation has been observed between these parameters and the poor metabolic control in DM1 patients.

Keywords: type 1 diabetes, magnesium, inflammation, oxidative stress

Magnesium (Mg) plays an essential role in a wide range of cellular reactions; it activates many and participates in synthesis of proteins and nucleic acids [1]. What is more, it is involved in metabolic pathways such as energy-dependent transport, glycolysis and phosphorylation [2]. Mg regulates ion channels and can act as the calcium (Ca) antagonist [3]. Moreover, Mg is involved in platelet aggregation and participates in vascular smooth muscle relaxation [4].

Mg deficiency is associated with oxidative stress and the inflammatory process [4, 5]. Epidemiological studies have shown that low concentrations of Mg in serum are related to the development
of atherosclerosis, and chronic cardiovascular diseases [6, 7]. The chronic, low grade inflammation and oxidative stress demonstrated in patients with type 1 diabetes (DM1) increases the risk of endothelial dysfunction and the development of late diabetic complications [8, 9]. Patients with DM1 showed lower levels of Mg concentration than healthy people.

DM1 is one of the main causes of Mg deficiency [10, 11]. It has been shown that hypomagnesaemia of DM1 patients could be one of the risk factors for the development of late diabetic complications [10, 12]. Therefore, the aim of the study was to evaluate the metabolic parameters, the concentration of oxidized low density lipoproteins (oxLDL) and the activity of platelet activating factor acetylhydrolase (PAF-AH) in the serum of DM1 patients with the concentration of Mg.

Material and methods

Study groups

The study was carried out in a group of 78 patients with DM1 (29 women, 49 men), aged 34.2 ± 6.0 years. This group had been under continuous observation at the Department of Internal Medicine and Diabetes in Poznan University of Medical Sciences from the onset of the disease. DM1 was diagnosed on the basis of typical symptoms, blood glucose concentration > 11.1 mmol/L, and C-peptide concentration < 0.5 μg/L [13]. From the onset of the disease all subjects were treated with intensive insulin therapy (IFTT) and were under observation once a year [14]. The study was carried out after a 10 ± 2 year observation period (starting from the diagnosis of the disease). The study group was divided according to the level of Mg concentration (< 0.7 mmol/L, group 1, n = 34; ≥ 0.7 mmol/L, group 2, n = 44). Cut-off value was established on the basis of the data taken from publications and the reference levels of the commercial test used for measuring the Mg concentration (0.70-0.98 mmol/L) [1, 16]. The control group concerned 24 healthy, not overweight subjects (12 women and 12 men), aged 30.6 ± 9.2 years. They were characterized by normal lipid profile and glucose concentration.

The study protocol was approved by the Ethics Committee of Poznan University of Medical Sciences. All the participants agreed to take part in the study, signing the informed written consent. Blood samples obtained in a fasting state were collected in tubes without anticoagulant. The samples clotted at room temperature and were then centrifuged at 2,000 g for 15 min to obtain serum. Before the analysis began all the samples were stored at -80°C.

Biochemical analyses

Standard laboratory procedures were applied to measure serum concentrations of glucose, lipid profile, apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I). Glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatography (HPLC) with the Variant Hemoglobin A1c Program (Bio-Rad Laboratories, Hercules, CA, USA) [17]. The concentration of Mg in serum was determined by the calmagite dye method (BioSystem, Spain). Briefly, the method is based on the reaction of Mg with 3-hydroxy-4[(2-hydroxy-5-methylphenyl)azo]-1-naphthalenesulfonic acid, to form a complex which absorbs light at 520 nm [16]. The concentration of Mg was read off from the standard curve.

oxLDL concentrations were measured using commercial enzyme-linked immunoabsorbent assay (Mercodia, Sweden). In the test, horse monoclonal antibody (mAbE6) was used against a conformational epitope of the apoB-100. This epitope is a consequence of substituting 60-lysine residues of apoB-100 for aldehyde, induced during the peroxidation of LDL [18]. Captured oxLDL was detected by a biotinylated antibody conjugated with the streptavidin-horseradish peroxidase (SA-HRP). 3,3′,5,5′-tetramethylbenzidine (TMB) was used to obtain the colorimetric reaction. The intensity of the color was measured spectrophotometrically at 450 nm. The concentration of oxLDL was read off from the standard curve.

PAF-AH activity was measured using colorimetric methods with a commercial kit, according to the instructions provided by the manufacturer (Cayman Chemical, USA). Serum was concentrated to one-fourth of its original volume for PAF-AF assay. The reaction was initiated by the addition of the substrate of enzyme - diheptanoyl Thio-PC. The absorbance was read at 405 nm after 1, 2, 3, and
4 minutes. PAF-AH activity was calculated using the formula provided with the method, and was expressed in μmol/min/ml.

**Statistical analysis**

Data are expressed as means ± SD or median and interquartile range. The studied parameters did not have a normal distribution. Therefore, nonparametric tests were used. Clinical parameters, Mg and oxLDL concentrations and PAF-AH activity were compared between patients with DM1 and the healthy subjects using the Mann Whitney test. The Kruskal-Wallis test was used for comparison of these parameters between patients with hypomagnesaemia (group 1) and those with reference levels of Mg in serum (group 2) and the control group. The Spearman correlation coefficient was used to test the strength of any associations between different variables. Multivariable regression analysis was performed as follows; Mg concentration *versus* parameters of poor metabolic control; FPG, PPG, HbA1c (model 1), *versus* lipid profile (model 2), *versus* oxLDL and PAF-AH (model 3). Moreover, another multivariable analysis was carried out using: oxLDL, PAF-AH, and HbA1c as the dependent variables. Odd ratios and confidence intervals were established by logit regression. The level of p < 0.05 was accepted as statistically significant.

**Results**

*Table 1* summarizes the clinical and metabolic information about the patients participating in the study.

<table>
<thead>
<tr>
<th></th>
<th>DM1 n=78</th>
<th>Control group n=24</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (F/M)</strong></td>
<td>29/49</td>
<td>12/12</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>34.2 ± 6.0 (32.0-38.0)</td>
<td>30.6 ± 9.2 (30.0-34.0)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diabetes duration (years)</strong></td>
<td>10.0 ± 2.0 (9.0-11.0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.6 ± 3.8 (23.0-27.0)</td>
<td>21.5 ± 1.0 (20.0-22.5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>FPG (mmol/L)</strong></td>
<td>9.2 ± 2.8 (7.6-9.8)</td>
<td>4.5 ± 0.5 (4.3-4.9)</td>
<td>p = 0.000</td>
</tr>
<tr>
<td><strong>PPG (mmol/L)</strong></td>
<td>9.0 ± 2.3 (7.0-11.2)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>8.1 ± 1.6 (7.1-9.5)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>TG (mmol/L)</strong></td>
<td>1.2 ± 0.7 (0.6-1.4)</td>
<td>1.0 ± 0.2 (0.95-1.15)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>TC (mmol/L)</strong></td>
<td>4.8 ± 1.0 (4.1-5.3)</td>
<td>4.0 ± 0.6 (3.6-4.6)</td>
<td>p = 0.0005</td>
</tr>
<tr>
<td><strong>LDL-C (mmol/L)</strong></td>
<td>2.9 ± 0.9 (2.3-3.4)</td>
<td>2.2 ± 0.6 (2.0-2.6)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td><strong>HDL-C (mmol/L)</strong></td>
<td>1.7 ± 0.4 (1.4-2.0)</td>
<td>1.3 ± 0.3 (1.2-1.4)</td>
<td>p = 0.0001</td>
</tr>
<tr>
<td><strong>Apo A-I (mg/dL)</strong></td>
<td>169.0 ± 25.7 (150.0-189.0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Apo B (mg/dL)</strong></td>
<td>83.2 ± 21.1 (69.0-95.5)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; FPG: fasting plasma glucose; PPG: postprandial plasma glucose; HbA1c: glycated hemoglobin A1c; TG: triglycerides; TC: total cholesterol level; LDL-C: LDL cholesterol; HDL-C: HDL cholesterol; apo A-I: apolipoprotein A I; apo B: apolipoprotein B.

NS: statistically non significant.
The concentration of Mg was lower in DMI patients in comparison with the control group [0.792 (0.625-0.835) vs 0.995 (0.915-1.125) mmol/L, p = 0.000]. DMI patients demonstrated increased PAF-AH activity and oxLDL concentration compared to the control group [0.0170 (0.014-0.0200) vs 0.0135 (0.0100-0.0160) μmol/min/mL, p = 0.04; 90.4 (78.9-100.0) U/L, p = 0.005, respectively].

Neither the Mg or oxLDL concentrations nor the PAF-AH activity were found to correlate with the serum concentration of metabolic variables. Moreover, multivariate regression analysis has shown that FPG, PPG, HbA1c do not influence the Mg concentration (β = -0.210, β = 0.230, β = -0.010, R² = 0.03). Similar results have been obtained for lipid profile: TC, LDL-C and HDL-C or apo-A1 and apo-B (β = -0.000, β = 0.012, β = -0.000, β = 0.047, β = 0.077, β = -0.130, R² = 0.0822) and oxLDL concentration, and PAF-AH activity (oxLDL; β = -0.41 and PAF-AH; β = 0.073, R² = 0.119) in DMI patients. However, it has been found that Mg concentration and PAF-AH activity influence oxLDL concentration independently (β = -0.33, β = 0.416, R² = 0.31). Logit analysis was conducted in order to refine the results of multivariate regression. It was observed that high oxLDL concentration diminishes the likelihood of the occurrence of reference levels of Mg [estimate = -0.067, p = 0.003, OR (95% CI) = 0.935 (0.893-0.978)].

**Table 2.** Characteristics of DM1 patients with Mg deficiency (group 1), patients with reference concentrations of Mg (group 2), and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 n = 34</th>
<th>Group 2 n = 44</th>
<th>Control group n = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>median (interquartile range)</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>12/22</td>
<td>17/27</td>
<td>12/12</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.6 ± 0.8</td>
<td>4.8 ± 1.0</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>4.6 (3.9-5.2)*</td>
<td>4.6 (4.4-5.4)*</td>
<td>4.0 (3.6-4.6)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.6 ± 0.4</td>
<td>2.9 ± 1.0</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2.6 (2.3-3.4)</td>
<td>2.9 (2.3-3.5)*</td>
<td>2.2 (2.0-2.6)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.7 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1.7 (1.5-2.0)*</td>
<td>1.7 (1.3-2.0)*</td>
<td>1.3 (1.2-1.4)</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>8.9 ± 2.3</td>
<td>8.5 ± 2.6</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>9.0 (7.2-11.5)*</td>
<td>9.0 (7.6-9.7)*</td>
<td>4.5 (4.3-4.9)</td>
</tr>
<tr>
<td>PPG (mmol/L)</td>
<td>8.8 ± 1.2</td>
<td>8.8 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8.9 (7.2-11.2)</td>
<td>8.0 (7.6-9.7)</td>
<td>-</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.4 ± 0.8</td>
<td>8.2 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8.4 (7.0-9.3)</td>
<td>8.1 (7.3-9.7)</td>
<td>-</td>
</tr>
</tbody>
</table>

FPG: fasting plasma glucose; PPG: postprandial plasma glucose; HbA1c: glycated hemoglobin A1c; TC: total cholesterol level; LDL-C: LDL cholesterol; HDL-C: HDL cholesterol.

* Statistically significant versus control group, p < 0.005.

Discussion
Our results confirm that Mg concentration in the serum of DM1 patients is lower in comparison to healthy people [11, 19]. However, no relation was observed between Mg concentration and poor metabolic control in DM1 patients, which is contrary to the results of other authors [10, 12, 20]. We also found that the DM1 patients with low serum Mg showed higher oxLDL concentrations and higher PAF-AH activity in comparison to the control group. Moreover, the results of logit regression confirmed the relationship between low Mg concentration and the oxLDL concentration in

The metabolic parameters were compared between patients with hypomagnesaemia (group 1), patients with reference levels of Mg (group 2) and the control group. As shown in table 2, no differences were observed between group 1 and group 2 with regard to metabolic control and serum lipid profile.

The concentration of Mg was lower in group 1 in comparison to group 2, and controls [0.623 (0.599-0.662) vs 0.830 (0.813-0.889) vs 0.995 (0.915-1.125) mmol/L, p < 0.05, respectively].

**Figures 1 and 2** respectively, show the highest oxLDL concentration and PAF-AH activity in group 1 in comparison to group 2 and the healthy subjects.
patients with DM1. Therefore, oxidative stress in patients with DM1 seems to be strongly associated with low Mg status.

OxLDL is formed during chemical changes caused by radical-mediated reactions. It leads to the production of hydroperoxides and reactive aldehydes [21]. High oxLDL concentration in DM1 patients with hypomagnesaemia indicates an association between Mg concentration and oxidative stress as well as a decrease in antioxidant concentrations. It has been found that Mg plays a protective role in the development of oxidative stress [22, 23]. It could be explained by the ability of Mg to regulate the activity of antioxidant enzymes. It has been shown that Mg intake reduces platelet cytochrome c oxidase activity [24]. The dysfunction of the enzyme may lead to overproduction of reactive oxygen species (ROS) [25]. Moreover, it has been found that Mg deficiency causes inhibition of the activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) [26]. In addition, Mg deficiency may influence LDL transport across the endothelial monolayer. It has been shown that Mg depletion intensifies LDL accumulation – the cause of higher oxidation of LDL enhancing the inflammation process and plaque development [27].

One should point out that high oxLDL concentration in the studied group seems to be inconsistent with the higher HDL concentration in the DM1 patients than in healthy people. These results suggest that the HDL of DM1 patients shows functional deficiency, even if the concentration of HDL was within the reference level. It has been found that DM1 patients demonstrated lower activity of paraoxonase 1 (PON1) than the healthy subjects [28]. This enzyme associated with HDL shows an anti-inflammatory capacity and reduces the development of atherosclerosis [29, 30]. Therefore, the results suggest that the high concentration of HDL is not sufficient for protection against LDL oxidation and atherosclerosis development in DM1.

It has been found that oxLDL stimulates the vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells, increasing the infiltration of monocytes into the vascular wall and stimulating the
macrophage proliferation [21]. In addition, oxLDL could lead to necrosis of vascular cells [31]. Therefore, this indicates that Mg deficiency increases oxidative damage in patients with DM1.

Higher activity of PAF-AH in DM1 patients with hypomagnesaemia in comparison to patients with normal Mg concentrations is further proof of the relation between the Mg deficiency, the increase in oxidative stress, and the chronic low grade inflammation in DM1 patients. PAF-AH hydrolyzes the ester bond at the sn-2 position of phospholipids, produces lysophosphatidylcholine (lyso-PC) and free fatty acids (FA) or oxidized fatty acids (oxFA). Lyso-PC increases adhesion molecule production and growth factor contributing to monocyte migration into the vascular wall [32]. Moreover, lyso-PC suppresses the production and the release of nitric oxide [33]. It has been shown that inhibition of PAF-AH activity diminishes plaque development [34]. PAF-AH also releases arachidonic acid; it may generate eicosanoids and leukotriens enhancing inflammatory response [35]. Therefore, higher activity of PAF-AH in the patients with hypomagnesaemia could contribute to inflammation development in DM1.

In conclusion, in the present study we show that the increase in oxLDL concentration and PAF-AH activity are related to low serum Mg concentration however, no relation was observed between these parameters and poor metabolic control in DM1 patients.

Acknowledgments

We wish to thank Mr. Chris Campbell for his help with the English editing of this text. We also would like to thank Professor Maria Iskra for critically reviewing the manuscript.

Financial support and disclosure

This work was supported by a PhD grant from the Polish Ministry of Science and Higher Education (No 402 476337).

None of the authors has any conflict of interest to disclose.
MAGNESIUM AND oxLDL CONCENTRATIONS AND PAF-AH ACTIVITY IN TYPE 1 DIABETES

References


