Plasma matrix metalloproteinase 9 correlates with disorders of brain magnesium homeostasis in patients undergoing coronary artery bypass surgery

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Abstract. Background. Changes in plasma matrix metalloproteinase 9 (MMP-9) concentrations and parallel changes in brain magnesium homeostasis have not been examined in cardiac surgery patients. The purpose of the present study was to analyse these relationships in patients undergoing coronary artery bypass surgery (CABG) with extracorporeal circulation (ECC). Additionally, the effect of volatile anaesthetics was considered. Patients and methods. Adult patients undergoing CABG with ECC under general anaesthesia were studied. Plasma MMP-9 and total (tMg) and ionized (iMg) magnesium concentrations were measured during surgery and during the early postoperative period. The plasma arteriovenous (a-v) tMg and iMg differences in the brain circulation were considered to be markers for brain magnesium homeostasis. The Mini-Mental State Examination test and computer tomography were used to diagnose postoperative neuropsychological disorders (PNPDs). Results. In total, 92 patients were examined. PNPDs were noted in 17 cases. Cardiac surgery resulted in increased plasma levels of MMP-9. The highest MMP-9 concentrations were observed in patients with PNPDs. MMP-9 concentrations strongly correlated with a-v tMg and a-v iMg differences. Compared with arterial measurements, venous tMg and iMg concentrations were higher during and immediately after surgery and lower during the early postoperative period. The most severe differences in a-v tMg and iMg were noted in patients with PNPDs. Conclusion. 1. Cardiac surgery resulted in an increase in plasma MMP-9 concentrations. 2. This increase in MMP-9 was significantly greater in patients with PNPDs. 3. The plasma MMP-9 concentration was correlated with disorders of brain Mg homeostasis. Key words: magnesium, matrix metalloproteinase 9, neuropsychological disorders, cardiac surgery, CABG, extracorporeal circulation

Metalloproteinases comprise a large family of proteolytic enzymes that include matrix metalloproteinases (MMPs) and proteins with a disintegrin and metalloproteinase domain [1]. Metalloproteinases play a crucial role in the activation of growth factors and death receptors as well as in neuroinflammation. They increase the permeability of the blood-brain barrier (BBB) as a result of
hypoxic-ischaemic processes, infections or multiple sclerosis [1-3]. Matrix metalloproteinases are divided into the following five main subgroups: collagenases, stromelysins, gelatinases, matrixins and membrane type matrix metalloproteinases [2, 3]. The two main gelatinases are matrix metalloproteinase-2 (gelatinase A, which is also known as MMP-2) and matrix metalloproteinase-9 (gelatinase B, also known as MMP-9). Uncontrolled gelatinase activity has been shown to be responsible for the degradation of collagen IV, fibronectin, laminin, proteoglycans and other critical extracellular matrix proteins [3, 4]. MMP-2 is a protease with a molecular weight of 72 kDa that is present in brain tissue and cerebrospinal fluid [5]. MMP-9 is produced by endothelial cells, microglia and astrocytes and has a molecular weight of 92kDa. Normally, MMP-9 is present at low levels and its plasma concentrations range from 15 to 100 ng/mL [2, 3, 5]. An increase in MMP-9 concentration has been observed after stroke and traumatic brain injury and during Alzheimer's disease, Guillain-Barre syndrome, bacterial meningitis, demyelization polyneuropathies and other pathologies [3, 4, 6, 7]. It has been documented that active MMP-9 digests the extracellular matrix, basal lamina and junctions between endothelial cells [6, 8, 9]. This activity under pathological conditions results in the degradation of the extracellular matrix, which leads to increased BBB permeability, neuroinflammation and brain oedema. Therefore, the plasma MMP-9 concentration is considered to be a sensitive marker of brain injury.

Magnesium (Mg) is one of the most important ions in the brain. It plays a crucial role in cell functions such as protein synthesis, regulation of calcium transport and accumulation, intracellular energy metabolism and preservation of membrane integrity [10]. Its brain concentrations are regulated by active BBB transport, and brain magnesium deficiency may result in a variety of neuropsychological disorders [11-14]. Several experimental studies have demonstrated a significant decline in brain magnesium after brain injury [14-16]. Moreover, the decrease in brain magnesium content is correlated with the severity of the brain injury and neurologic outcome [15, 19]. Neuronal decline is characteristic of the secondary biochemical events associated with brain injury and is strongly dependent on the plasma total and ionised magnesium concentrations (tMg and iMg, respectively) [17, 18]. Therefore, many authors have recommended the supplementation of magnesium immediately after brain injury [19-21]. Furthermore, neurological disorders are not only diagnosed after brain trauma, but cardiac surgery with ECC may also result in neuropsychological dysfunctions [22, 23]. These forms of brain damage may occur through intraoperative micro- and macroembolisms, rapid circulation disorders, normovolemic haemodilution, inflammatory responses and severe electrolyte disorders. Many authors have described severe hypomagnesaemia during and after cardiac surgery with ECC [21, 24, 25]. Some studies have examined the correlation between magnesium and neuropsychological outcomes in patients undergoing coronary artery bypass surgery (CABG) with ECC.

Patients and methods

This study was approved by the Committee for Bioethics at the Medical University of Lublin and informed consent was obtained from all patients. Patients scheduled for elective CABG due to stable angina pectoris were examined. The exclusion criteria included the following: any neurological disease or history of neurological disorders, head surgery or severe head trauma, significant carotid artery stenosis, any chronic respiratory disease, serious endocrine diseases, chronic renal insufficiency, unstable angina pectoris, chronic renal failure and a EuroScore higher than 8.

One day before surgery, all patients received oral lorazepam (Lorafen, Polfa, Rzeszow, Poland) (2 mg). One hour before the induction of anaesthesia all patients received intramuscular morphine hydrochloride (Morphicum hydrochloricum, Warsaw, Polfa, Poland) (0.1 mg/kg body wt) with midazolam (Sopodorm, Polfa, Rzeszow, Poland) (0.01 mg/kg body wt).

Anaesthesia and surgery

Before the induction of anaesthesia, all patients were routinely monitored with respect to electrocardiography (leads II, III, V5, aVF, aVR and eVL), arterial and central venous pressure and pulmonary arterial pressure.
The induction of anaesthesia was performed with fentanyl (Fentanyl, Polfa, Warsaw, Poland) (0.01-0.02 mg/kg body wt), midazolam (0.05-0.1 mg/kg body wt) and etomidate (Etomidate, Braun, Melsungen, Germany) (0.1-0.5 mg/kg). Muscle relaxation was induced by injecting a single dose (0.08-0.1 mg/kg body wt) of pancuronium (Pavlon, Pancuronium, Jella, Jelenia Gora, Poland). After tracheal intubation, mechanical ventilation with a mixture of air and oxygen (60% and 40%, respectively) was provided. All patients were ventilated using intermittent positive pressure ventilation (IPPV) with tidal volume (6-7 mL/kg body wt) and respiratory rate (9 per min). The parameters were adjusted to achieve normocapnia, which was controlled by gas analysis. The anaesthesia was maintained throughout the procedure using a midazolam-fentanyl infusion.

Intraoperative hypertension was treated with a volatile anaesthetic [isoflurane (Forane, Baxter, Guyana, USA) or sevoflurane (Sevorane, Abbot, Berks, UK) at a dose of 0.5-1% vol]. The type of volatile anaesthetic was randomly assigned. In patients not responding to anaesthesia, a single intravenous dose of urapidil was used (Ebrantil, Altana, Germany). Tachycardia was treated with beta-blockers.

After the induction of anaesthesia but before surgery, a Swan-Ganz catheter (ARROW, USA) was inserted via the left internal jugular vein. The thermodilution technique utilising a 10 ml bolus of ice-cold saline was used for cardiac output measurements. Pulmonary and systemic haemodynamic parameters were measured during the surgery and early postoperative period.

Before ECC, heparinum sulfuricum (Heparin, Polfa, Warsaw, Poland) was used at a dose of 3 mg/kg body wt, and the activated clotting time was controlled up to 400 sec. For ECC, standard cannulation of the ascending aorta and inferior vena cava was performed through the right atrium. During ECC, circulation and ventilation were maintained with the heart-lung machine S III (Stöckert, Munich, Germany). The machine priming fluid consisted of 1,000 mL of Ringer’s solution (Ringer, Polfa Kutno, Poland), 500 mL of 6% hydroxyethylated starch (Voluven, Freseniuss-Kabi, Kutno, Poland), 250 mL of 20% mannitol (Mannitol, Freseniuss-Kabi, Kutno, Poland), 20 mL of sodium hydroxycarbonate (Natrium bicarbonatum, Polfarma Starogard Gdański, Poland) and 75 mg of heparinum sulfuricum. Cardiopulmonary bypass was instituted at a pulsating flow rate of 2.4 L/min per m² of body surface area (BSA). After traditional aortic clamping, myocardial viability was preserved with antegrade hyperkalaemic warm blood cardiopleagia. During mild hypothermic ECC, the mean arterial pressure, haematoctrit and gasometric parameters as well as the lactate, sodium and potassium levels were measured. Distal anastomoses were made during cardioplaegic arrest, whereas proximal ones were performed with resumed perfusion and a side-biting clamp. In all cases, the separation from the heart-lung machine was uneventful and intra-aortal counterpulsation was not necessary. After the termination of ECC, some patients received an infusion of dopamine hydrochloride (Dopaminum, Polfa, Warsaw, Poland) or dobutamine hydrochloride (Dobutamin, Hexal, Wassenburg, Germany) at doses adjusted for their clinical condition (3-15 μg/kg per min and 3-9 μg/kg per min, respectively). The effect of heparin was reversed by an adequate dose of protamine sulphate (Protaminum sulfuricum, Biomed, Warsaw, Poland) (1 mg protamine per 1 mg of heparin). During surgery and in the early postoperative period, patients received a supplemental mixture of potassium chloride and magnesium sulphate.

After surgery, patients were sent to the postoperative intensive care unit (PICU). All of them were ventilated using synchronised intermittent mandatory ventilation (SIMV) with pressure support. Patients were extubated 8 to 12 hrs after surgery and transferred from the PICU between the second and third postoperative day.

After the induction of anaesthesia and until the beginning of ECC, 500 mL of gelatine (Gelafundin, Braun, Malsungen, Germany) was infused. After ECC, haematologic parameters were monitored. None of the patients required massive fluid resuscitation and the type and dose of administered fluids depended on the patients’ haemodynamic status. Insufficiency of intravascular fluids in the early postoperative period was treated by supplementation with gelatine preparations or electrolyte fluids (PWE, Ringer - Polfa, Kutno, Poland).

Study protocol and patient distribution

Observations were conducted at the following five time points: 1) after the induction of anaesthesia and before surgery, 2) 10 min after the disconnection of the heart-lung machine, 3) after completion of the procedure but before the patient was sent to the postoperative intensive care unit, 4) the morning of the first postoperative day and 5) the morning of the second postoperative day. The Mini-Mental State Examination test (MMSE) and computer tomography were used for diagnosis of postoperative...
neuropsychological disorders (PNPDs). The MMSE was performed one day prior to surgery and on the third postoperative day. According to the MMSE, neuropsychological status was defined in points (from 1 to 30). A score lower than 9 was considered severe PNPDs, values between 10 and 23 meant slight cognitive disorders and higher than 24 was normal. Computer tomography was performed in all cases where there was any suspicion of a severe postoperative neuropsychological pathology.

Blood samples were collected from the radial artery for MMP-9, tMg and iMg measurements. The retrograde right jugular vein was cannulated (the jugular vein bulb) for plasma venous tMg and iMg determinations. The arteriovenous (a-v) magnesium differences were considered as markers for brain magnesium homeostasis. Blood samples were immediately centrifuged (2,500 r/min) and the resulting serum was frozen at -20°C. Next, xylidine blue was added to each of the defrosted samples. Serum tMg concentrations were determined by spectrophotometric methods with ultraviolet light at a wavelength of 520 nm (Spectrophotometer SPECORD M40, Zeiss, Jena, Germany). An ion-selective Mg²⁺ electrode (measurement range 0.2-3.0 mmol/L, accuracy ± 3%, precision within run 2% (CV) or 0.04 mmol/L (SD), selectivity logKMgNa = -3.0; logKMgK = -2.2; logKMGCa = -0.2) and analyzer Microlyte 6 (Thermo Konelab, Vantaa, Finland) were used for iMg measurements.

The enzyme-linked immunosorbent assay (ELISA) method was used to determine serum active MMP-9 concentrations. The defrosted samples were centrifuged (50,000 r/min). The plasma was buffered and diluted to 1:100. Samples were incubated in microplate wells with a specific monoclonal antibody for 2 hrs (DMP 900 R&D). Next, the polyclonal antibodies for MMP-9 were added. The reaction was stopped by the addition of an acidic sulphuric solu tion. The product was measured spectrophotometrically at 450 nm.

Statistics

Means and standard deviations (SD) were calculated for parametric data. The value at time point 1 was regarded as baseline. Categorical variables were compared using the χ² and Fisher exact test, and the Yates correction was applied. Student’s unpaired t-test was used to analyse variables with a normal distribution. Non-parametric data were statistically analysed using the Wilcoxon signed-rank test and the Kruskal-Wallis ANOVA test for initial detection of differences. Dunnett’s multiple comparison post-hoc test and Spearman’s rank correlation test were used for inter-point and inter-group comparisons. Additionally, the Spearman’s rank correlation test was used for the overall analysis. P < 0.05 was considered significant. A preliminary estimate of sample size was based on expected changes in plasma MMP-9 concentration at consecutive time points. With a type I error of 0.05 and a type II error of 0.2, the required sample size was 21-25 patients. The dropout rate was estimated at 10%; thus, a minimum of 29 patients had to be examined. The sample size was determined by Statistica 9 software. The power of all statistical tests was determined by G^Power software (1-β).

Results

One hundred and twenty-eight adult patients were examined between January 2006 and December 2008. Thirty-six cases were excluded due to serious postoperative haemodynamic disorders (i.e. postoperative low cardiac output syndrome due to atrial fibrillation or other arrhythmias, perioperative myocardial infarction and use of intra-aortic counterpulsion) and/or postoperative bleeding requiring reoperation.

Finally, 92 adult patients (19 women and 73 men) aged 66 ± 6 years undergoing elective CABG with ECC were included. The mean duration of anaesthesia was 255 ± 29 min, surgery was 201 ± 25 min, ECC was 115 ± 31 min and aorta clamping was 59 ± 19 min. Extracorporeal circulation and distal anastomoses were performed under mild hypothermia (table 1). All distal anastomoses were constructed during a single period of total aorta clamping; proximal anastomoses were constructed with partial clamping of the aorta. The mean arterial pressure was between 45 and 105 mmHg during ECC. An uncomplicated postoperative period (UPP) was noted in 75 patients (82%) and PNPDs were observed in 17 patients (18%). Various types of serious psychoses were noted in 12 patients and stroke was noted in 5 cases. In the study population, the mean value of MMSE was 27.63 ± 1.68 before surgery and 19.27 ± 7.57 on the third postoperative day.

In the study population, the baseline values of MMP-9, a-v tMg and a-v iMg were 61.66 ± 15.6 ng/mL, -0.01 ± 0.18 mmol/L and 0.0 ± 0.11 mmol/L, respectively. Cardiac surgery resulted in an increase in MMP-9 (figure 1), and higher plasma MMP-9 concentrations were observed in patients with PNPDs (table 2). Ten minutes after ECC and just after surgery, plasma tMg and iMg concentrations were higher in the jugular vein than in the artery. Other-
wise, plasma tMg and iMg concentrations were higher in the artery than in the jugular vein on the early postoperative days (figure 2). Plasma a–v tMg and iMg were more severely changed in patients with PNPDs.

In the study population, there was a strong negative overall correlation between plasma MMP-9 and a–v tMg as well as between MMP-9 and a–v iMg concentrations ($r = -0.57, p < 0.001, 1-\beta = 0.95$ and $r = -0.56, p < 0.001, 1-\beta = 0.95$, respectively). Similarly, there was a strong overall correlation between MMP-9 and a–v tMg and between MMP-9 and a–v iMg concentrations in patients with UPP.

Table 1. Demographic data of the studied patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Value</th>
<th>Age (yrs)</th>
<th>Duration of (min)</th>
<th>BMI (kg/m²)</th>
<th>BSA (m²)</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studied population</td>
<td>92</td>
<td>Mean</td>
<td>66</td>
<td>255</td>
<td>201</td>
<td>115</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>6</td>
<td>29</td>
<td>25</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>Group UPP</td>
<td>75</td>
<td>Mean</td>
<td>66</td>
<td>256</td>
<td>202</td>
<td>119</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>6.21</td>
<td>30</td>
<td>26</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>Group PNPD</td>
<td>17</td>
<td>Mean</td>
<td>66</td>
<td>252</td>
<td>195</td>
<td>98</td>
<td>53</td>
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<td></td>
<td></td>
<td>SD</td>
<td>4.82</td>
<td>23</td>
<td>18</td>
<td>22</td>
<td>16</td>
</tr>
</tbody>
</table>

UPP: patients with an uncomplicated postoperative period; PNPDs: patients with postoperative neuropsychological disorders; ECC: extracorporeal circulation; AC: aorta clamping; BMI: body mass index; BSA: body mass surface.

Figure 1. Changes in plasma matrix metalloproteinase 9 concentrations within the study population.

* $p < 0.05$, *** $p < 0.001$ compared with time point 1 (Wilcoxon signed-rank test).
Median, 25%-75%, Min. - Max. Time points: (1) after the induction of anaesthesia and before surgery, (2) 10 min after the disconnection of the heart-lung machine, (3) after completion of the procedure but before the patient was sent to the postoperative intensive care unit (PICU), (4) the morning of the first postoperative day and (5) the morning of the second postoperative day.
Table 2. Changes in plasma matrix metalloproteinase 9, total and ionised magnesium concentrations (tMg and iMg, respectively), in patients with an uncomplicated postoperative period (UPP) and with postoperative neuropsychological disorders (PNPDs).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Value</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Intergroup differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>UPP</td>
<td>Quartile 1</td>
<td>55.30</td>
<td>80.29</td>
<td>79.82</td>
<td>35.83</td>
<td>25.90</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>65.42</td>
<td>82.49***</td>
<td>82.28***</td>
<td>63.55**</td>
<td>59.78***</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>72.04</td>
<td>88.49</td>
<td>89.10</td>
<td>71.48</td>
<td>71.69</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>PNPD</td>
<td>Quartile 1</td>
<td>39.24</td>
<td>90.73</td>
<td>95.46</td>
<td>81.10</td>
<td>76.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>63.12</td>
<td>97.57***</td>
<td>113.97***</td>
<td>112.46***</td>
<td>97.43*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>75.41</td>
<td>100.34</td>
<td>186.53</td>
<td>175.40</td>
<td>124.31</td>
<td></td>
</tr>
<tr>
<td>tMg (mmol/L)</td>
<td>UPP</td>
<td>Quartile 1</td>
<td>-0.06</td>
<td>-0.15</td>
<td>-0.15</td>
<td>0.06</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>-0.01</td>
<td>-0.04***</td>
<td>-0.04***</td>
<td>0.10***</td>
<td>0.10***</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>0.05</td>
<td>0.01</td>
<td>0.00</td>
<td>0.15</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PNPD</td>
<td>Quartile 1</td>
<td>-0.04</td>
<td>-0.29</td>
<td>-0.44</td>
<td>-0.16</td>
<td>-0.01</td>
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<tr>
<td></td>
<td></td>
<td>Median</td>
<td>0.03</td>
<td>-0.18***</td>
<td>-0.36***</td>
<td>-0.05</td>
<td>0.15*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>0.04</td>
<td>-0.12</td>
<td>-0.27</td>
<td>0.11</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>iMg (mmol/L)</td>
<td>UPP</td>
<td>Quartile 1</td>
<td>-0.03</td>
<td>-0.09</td>
<td>-0.09</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>-0.01</td>
<td>-0.02***</td>
<td>-0.02***</td>
<td>0.06***</td>
<td>0.06***</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>0.04</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.09</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PNPD</td>
<td>Quartile 1</td>
<td>-0.02</td>
<td>-0.17</td>
<td>-0.25</td>
<td>-0.13</td>
<td>-0.01</td>
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<tr>
<td></td>
<td></td>
<td>Median</td>
<td>0.02</td>
<td>-0.11***</td>
<td>-0.20***</td>
<td>-0.05</td>
<td>0.07</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Quartile 3</td>
<td>0.02</td>
<td>-0.07</td>
<td>-0.15</td>
<td>0.07</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

Time points: (1) after the induction of anaesthesia and before surgery, (2) 10 min after the disconnection of the heart-lung machine, (3) after completion of the procedure but before the patient was sent to the postoperative intensive care unit (PICU), (4) the morning of the first postoperative day and (5) the morning of the second postoperative day.

NS: non significant. * p < 0.05, ** p < 0.01, *** p < 0.001 compared with time point 1 (Wilcoxon and U-Mann-Whitney U tests).

There were strong correlations between MMP-9 and a-v tMg as well as between MMP-9 and iMg concentrations at time points 2 and 3 (r = -0.57, p < 0.001, 1-β = 0.95 and r = -0.57, p < 0.001, 1-β = 0.95, respectively). There was also a correlation between MMP-9 and a-v tMg and between MMP-9 and a-v iMg in patients with PNPDs (r = -0.6, p < 0.001, 1-β = 0.96 and r = -0.64, p < 0.001, 1-β = 0.96, respectively).

Discussion

The present study documented that CABG with ECC resulted in an elevation of plasma MMP-9 concentrations. The increase in MMP-9 concentration was greater in patients with PNPDs. Moreover, our findings demonstrated an increase in venous plasma magnesium concentrations just after ECC and surgery compared with arterial concentrations. The opposite situation was noted during the first and second postoperative days, when plasma arterial magnesium concentrations were higher than in the
These magnesium alterations were negatively correlated with MMP-9 levels. This is the first study to examine correlations between MMP-9 and disturbances in brain tMg and iMg homeostasis in patients who underwent elective CABG with ECC.

Examination of tMg and iMg levels in the brain circulation was one of the most important purposes of our study. Our findings suggested that ECC reduced the magnesium content in the brain. Several authors have documented a significant decrease in plasma magnesium concentrations during and just after ECC [24, 26-28]. It has been shown that such disorders result from intraoperative blood dilution, increased loss of magnesium in the urine or ineffective magnesium supplementation. However, changes in tMg and iMg associated with disorders of brain magnesium homeostasis have not been documented. Precise observations of changes in brain magnesium content in patients undergoing cardiac surgery have not been technically feasible, and the analysis of arteriovenous differences has shown only general disturbances in brain magnesium. Decreased brain magnesium has been shown to be characteristic of ischaemic/traumatic brain injury [12-19]. Many experimental and clinical studies have demonstrated significant decreases in brain and plasma magnesium concentrations following traumatic/ischaemic brain injury [13, 16, 29]. Moreover, the degree of decrease in brain magnesium has been shown to correlate with post-traumatic/ischaemic neurological outcomes [13, 17, 21]. Similarly, the most severe disturbances in magnesium were noted in patients with PNPDs. Therefore, our findings suggest a neurodestructive effect of ECC.

During the early postoperative period, plasma magnesium concentrations were higher in the arterial circulation. These differences suggested that there was a deficiency of magnesium in the brain after ECC. Some investigators have documented more severe neurological outcomes in patients who have received extra supplementation of magnesium during cardiac surgery [21]. These studies noted better short-term memory, a significant
reduction of cerebellar complications and a marked return of primitive reflexes in patients receiving magnesium. Notably, magnesium supplementation reduced plasma S100β protein levels in cardiac surgery patients [30]. Additionally, other authors have reported a neuroprotective effect of magnesium in patients after traumatic/ischaemic events [17, 20, 31]. For this reason, careful clinical observation and correction of plasma magnesium disorders has been recommended in patients after brain injury [31-33].

Several investigators have documented an increase in plasma MMP-9 as a result of brain injury [8, 9, 34-38]. Most of these studies have shown an increase in BBB permeability mediated by MMP-9 that leads to vasogenic oedema, haemorrhage and cell death [8, 9, 36, 38]. The degradation of components of the basal lamina such as laminins, collagen IV or fibronectin predisposed weakened brain microvessels to rupture and transformed an ischaemic brain injury into a haemorrhagic episode [8, 39]. Rosell and colleagues documented the crucial role of MMP-9 in haemorrhagic transformation [39]. They observed a higher MMP-9 concentration in infarcted and/or haemorrhagic areas, higher levels of the activated form of MMP-9 in haemorrhagic areas, a higher MMP-9 content in the microvascular endothelium and more severe degradation of collagen IV in vessels with neutrophil and erythrocyte extravasation. Moreover, a high level of MMP-9 was noted not only in the infarcted tissue but also in peri-infarcted areas, which suggested that MMP-9 contributed to infarct progression [40]. Accordingly, several authors suggested that plasma MMP-9 concentrations were associated with the severity of stroke, stroke size and neurological deficits [41].

Similarly, we observed higher plasma MMP-9 concentrations in patients with PNPDs, and the relatively high dispersion rate led to the speculation that MMP-9 levels depended on the degree of brain injury. However, the small sample of patients with PNPDs significantly limited the power of statistical analysis, and such relationships should be confirmed in further studies.

Plasma MMP-9 concentrations were negatively correlated with total and ionised brain magnesium levels, and these correlations were slightly stronger in patients with PNPDs. Moreover, MMP-9 levels were strongly and negatively correlated with the magnesium concentration just after ECC surgery and on the morning of the first postoperative day. Yet, the correlation between MMP-9 and magnesium has been not previously documented. Pages and colleagues documented the presence of an active form of MMP-9 in the vascular walls of magnesium-deficient mice [42]. More important, they found the inactive form of MMP-9 in mice without magnesium disorders. They postulated that magnesium played a key role as an endogenous tissue inhibitor of metalloproteinase activity; such an effect could explain the negative correlation we observed between MMP-9 and magnesium. However, interactions between magnesium and MMP-9 or other tissue inhibitors of metalloproteinases have not been precisely documented.

The negative correlation between magnesium and MMP-9 may be a result of N-methyl-D-aspartate (NMDA) receptor activity because magnesium is a non-competitive NMDA receptor antagonist [43]. Glutamate activation of the NMDA receptor leads to increased cellular calcium, which activates a complex biochemical cascade that includes protein kinase activation, calpain-induced cytoskeletal breakdown, DNA fragmentation and reactive oxygen species accumulation [43-45]. After prolonged activation, neurons become damaged and subsequently die. More important, such excitotoxic processes are some of the most important autodestructive responses during the early posttraumatic period after brain injury. The activation of NMDA receptors is reversibly modulated by MMP-9 [46]. However, the mechanism underlying MMP-9's regulation of the NMDA receptor is not clear. Some investigators have postulated that MMP-9 modulation of NMDA receptors requires proteolytic activity of MMP-9 and is not dependent on protein–protein interactions [46, 47]. Therefore, we can assume that changes in brain magnesium content can affect MMP-9; nevertheless, the interaction between iMg and tMg remains to be elucidated.

Despite the promise of the novel findings of our study, a few limitations should be discussed. First, this study considered that differences between arteriovenous plasma magnesium concentrations were reflective of disordered brain magnesium homeostasis. However, the analysis showed only a general tendency towards significant changes in brain magnesium. For direct measurements of disordered brain magnesium homeostasis, analysis of the cerebrospinal fluid (CSF) magnesium concentration should be performed. Significant increases in CSF magnesium were observed after serious traumatic brain injury and correlated with poor neurological outcomes [29]. Nevertheless, the determination of CSF magnesium concentration as a marker of posttraumatic outcome is still controversial. Some
authors have not observed an increase in CSF magnesium after traumatic brain injury [48]. It was speculated that increased CSF magnesium resulted from disruption of the BBB after severe trauma [29]. In the present study, PNPDs were noted in 17 patients. Therefore, the arteriovenous plasma magnesium differences could be a relatively credible marker of disordered brain magnesium homeostasis, particularly in patients with UPP.

The analysis of arterial MMP-9 concentrations was another important limitation of our study. However, the observed arteriovenous MMP-9 differences in the brain circulation were credible, and previous studies demonstrated that cardiac ischaemia, myocardial infarction and asthma result in increased levels of MMP-9 [49-51]. Moreover, increased plasma MMP-9 has been associated with left ventricular remodelling after myocardial infarct [52]. This process was also observed after CABG, and elevated levels of MMP-9 have been noted after ECC [53-55]. These changes in MMP-9 have been shown to contribute to ECC-induced inflammatory reactions [55], which are some of the most important reactions in post-ECC brain injury. Therefore, we suggested that elevated concentrations of MMP-9 affected patient outcome in neuronal magnesium disorders.

The lack of correlations between plasma MMP-9 concentrations and perioperative treatment was also a limitation of our study. Several investigators have shown lower plasma MMP-9 concentrations in patients during statin therapy [56, 57]. A six-month treatment with simvastatin significantly reduced MMP-9 in patients with atherosclerotic cerebral infarction [57]. In our study, the preoperative administration of statin also reduced plasma MMP-9 concentrations. Therefore, the effect of preoperative treatment on MMP-9 dysregulation (as well as during disordered brain magnesium homeostasis) requires further analysis.

Finally, we showed that ECC increased plasma MMP-9 concentrations, which negatively correlated with arteriovenous tMg and iMg concentrations. More important, these correlations were stronger in patients with PNPDs. These changes in both magnesium forms were associated with effects of ECC on brain magnesium.

Disclosure

None of the authors has any conflict of interest or financial support to disclose.

References


