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Matrix metalloproteinases and left ventricular function and structure in spinal cord injured subjects

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ABSTRACT

Subjects with spinal cord injury (SCI) exhibit impaired left ventricular (LV) diastolic function, which has been reported to be attenuated by regular physical activity. This study investigated the relationship between circulating matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) and echocardiographic parameters in SCI subjects and the role of physical activity in this regard. Forty-two men with SCI [19 sedentary (S-SCI) and 23 physically-active (PA-SCI)] were evaluated by clinical, anthropometric, laboratory, and echocardiographic analysis. Plasmatic pro-MMP-2, MMP-2, MMP-8, pro-MMP-9, MMP-9, TIMP-1 and TIMP-2 levels were determined by enzyme-linked immunosorbent assay and zymography. PA-SCI subjects presented lower pro-MMP-2 and pro-MMP-2/TIMP-2 levels and improved markers of LV diastolic function (lower E/Em and higher Em and E/A values) than S-SCI ones. Bivariate analysis showed that pro-MMP-2 correlated inversely with Em and directly with E/Em, while MMP-9 correlated directly with LV mass index and LV end-diastolic diameter in the whole sample. Following multiple regression analysis, pro-MMP-2, but not physical activity, remained associated with Em, while MMP-9 was associated with LV mass index in the whole sample. These findings suggest differing roles for MMPs in LV structure and function regulation and an interaction among pro-MMP-2, diastolic function and physical activity in SCI subjects.

1. Introduction

Decreases in left ventricular (LV) diastolic function are consistently associated with increased risk of cardiovascular events [1]. Subjects with spinal cord injury (SCI) exhibit worse LV diastolic function and higher cardiovascular risk in comparison with able-bodied individuals, independent of traditional cardiovascular risk factors [2–5]. Recently, regular physical activity was reported to be associated with improved LV diastolic function in SCI individuals, independent of variation in hemodynamic, metabolic and inflammatory variables [6]. Although these data suggest that physical inactivity plays a major role in the development of LV diastolic dysfunction in SCI subjects, the precise mechanisms that underlie this association remain uncertain.

Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade the extracellular matrix and have been involved in the pathophysiology of LV dysfunction and remodeling [7–9]. Elevated circulating concentrations of MMPs have been related to reduced LV diastolic function and alterations in LV structure in several clinical settings [10–12]. Conversely, regular physical activity was associated with decreased levels of MMPs and improved LV diastolic function in able-bodied subjects [13]. Therefore, the present report investigated the LV function and structure of physically active (PA-SCI) and sedentary (S-SCI) SCI subjects and evaluated the impact of MMPs and tissue inhibitors of MMPs (TIMPs) in this regard.

2. Material and methods

2.1. Subjects

A total of 42 men (19 S-SCI and 23 PA-SCI) with at least 2 y of SCI were cross-sectionally evaluated. S-SCI subjects were enrolled in the

Abbreviations: MMP, matrix metalloproteinase; PA-SCI, physically active subjects with spinal cord injury; S-SCI, sedentary subjects with spinal cord injury; SCI, spinal cord injury; TIMP, tissue inhibitors of matrix metalloproteinase.

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hospital of the University of Campinas and did not perform sports, recreational physical activity or labor that required physical effort. PA-SCI individuals comprised competing athletes that were regularly performing wheelchair rugby (n = 13), basketball (n = 9) and handball (n = 1) for at least 1 y. All athletes were enrolled in the School of Physical Education of the University of Campinas and had been training in average for 11.9 ± 1.4 h/wk for 4.4 ± 0.5 y. Exclusion criteria for all groups included diabetes mellitus, systemic hypertension, hyperlipidemia [14], current or past smoking, known coronary artery, cardiac or pulmonary disease, cancer, regular medical therapy and clinical evidence of active infection. SCI subjects presented no preserved motor function below the injury level. According to the American Spinal Injury Association Impairment Scale, 38 subjects were grade A and 4 individuals were grade B (2 S-SCI and 2 PA-SCI). Written informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved by the Human Research Ethics Committee of the University of Campinas.

2.2. Clinical, laboratory and hemodynamic data

Clinical data included information on the participants' age and injury duration. Body mass index was calculated as body weight divided by height squared. Blood samples were obtained in the morning after 12 h of fasting for analysis of glucose, lipid fractions and C-reactive protein. Office blood pressure was measured using validated digital oscillometric device with the subjects in the sitting position (Omron HEM-705CP, Omron Corp). Two readings were averaged and if they differed by more than 5 mm Hg, one additional measurement was performed and then averaged.

2.3. Matrix metalloproteinases analysis

Plasma samples were collected after 12 h of fasting and immediately frozen to −80 °C. Then commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate total plasmatic concentrations of MMP-2, MMP-8, MMP-9, TIMP-1 and TIMP-2 (R&D Systems). Previous studies have shown that not only the total circulating levels of MMP-2 and MMP-9, but also the plasmatic levels of the pro (precursor) forms of these MMPs might be related to LV remodeling [15]. To assess the expression of the pro-forms of MMP-2 and MMP-9, gelatin zymography was performed as previously described [16]. Briefly, plasma samples were electrophoresed on a 7% polyacrylamide gel containing 2 g/l gelatin. Gels were stained in 0.5% Coomassie blue R-250 and distained for 1-h in 40% methanol:10% acetic acid. MMP proteolytic activity was determined by densitometry analysis and the pro-forms of MMP-2 (pro-MMP-2) and MMP-9 (pro-MMP-9) were identified as bands at 72 and 92 kDa, respectively [17].

2.4. Echocardiography studies

Echocardiography studies were performed by a skilled physician on each subject in the sitting position with a Vivid 3 Pro apparatus (General Electric) equipped with a 2.5-MHz transducer, as previously described [6,18]. Cardiac dimensions were assessed according to the recommendations of the American Society of Echocardiography [19]. LV mass and LV geometric pattern were evaluated by LV mass index (LV mass/body surface area) and relative wall thickness (2 * posterior wall thickness/LV end-diastolic diameter). Tissue Doppler imaging estimated peak spectral longitudinal contraction (Sm), as well as initial (Em), and final (Am) diastolic velocities [6]. LV diastolic function was evaluated by peak early/atrial velocity ratio (E/A), Em and E/Em ratio, while LV systolic function was assessed by LV ejection fraction and Sm values. Stroke volume was generated from Doppler interrogation of transaortic flow at the aortic annular level and aortic cross-sectional area. Cardiac output was calculated as stroke volume × cardiac frequency, while peripheral vascular resistance was obtained by the formula: mean blood pressure / cardiac output. Intraobserver LV mass, E/Em ratio and Em variabilities were <6%, <6% and <7%, respectively.

2.5. Statistical analysis

Results were analyzed using SPSS 15.0™. Continuous normal and non-normal variables are presented as mean ± SEM and median (25–75th percentile), respectively. Based on previous studies [3,6], a sample size of at least 14 individuals in each group was considered suitable for detecting significant differences in LV diastolic function regarding values of alpha error = 0.05 and beta error = 0.80. However data collection was extended to the 19th and 23rd individuals in the S-SCI and PA-SCI groups, respectively. The Kolmogorov–Smirnov test was used to test for normal distribution of the variables. Log transformation was applied to skewed variables in order to use parametric tests. Differences in clinical, laboratory, hemodynamic and echocardiographic continuous variables were evaluated by unpaired *t*-test. χ^2 was used to compare categorical variables. Assessment of bivariate correlations between variables was examined using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient when categorical variables were included. Two-way analysis of covariance (ANCOVA) was used to assess intergroup differences in selected variables after adjustment for potential confounding variables. Linear regression analysis was used to evaluate the independent predictors of selected echocardiographic parameters. Variables that exhibited significant correlation at bivariate analysis were included as independent variables in regression analyses. Due to high collinearity, systolic and diastolic blood pressures were not included in a same multivariate model. In this regard, the blood pressure measurement exhibiting the higher correlation coefficient with the dependent variable was included in the model. A p-value < 0.05 was considered significant.

3. Results

3.1. Clinical and laboratory features and plasmatic levels of MMPs and TIMPs of studied subjects

Clinical, laboratory and hemodynamic features of enrolled subjects are presented in Table 1. The studied groups exhibited similar features, except for higher C-reactive protein levels in S-SCI individuals compared with the PA-SCI ones. Plasmatic levels of MMPs and TIMPs are presented in Table 2. S-SCI individuals showed higher pro-MMP-2 and pro-MMP-2/TIMP-2 ratio than PA-SCI subjects, while no further differences in other MMPs, TIMPs and MMP/TIMP ratios were detected between the studied groups. Furthermore, the differences in pro-MMP-2

Table 1
Clinical, hemodynamic and laboratory features of enrolled subjects.

Variable	Sedentary SCI (n = 19)	Physically active SCI (n = 23)	p
Age, y	33.7 ± 2.2	29.6 ± 1.2	NS
Time of injury, y	7.2 ± 1.1	9.2 ± 0.9	NS
Tetraplegic, n (%)	9 (47)	13 (57)	NS
Body mass index, kg/m^2	23.7 ± 0.9	22.3 ± 0.6	NS
Systolic blood pressure, mm Hg	105.1 ± 3.7	112.7 ± 4.8	NS
Diastolic blood pressure, mm Hg	68.2 ± 2.2	71.1 ± 3.0	NS
Heart rate, b.p.m.	73.7 ± 2.7	66.7 ± 2.6	NS
Cardiac output (l/min)	4.8 ± 0.3	4.8 ± 0.3	NS
PVR (dynes × s × cm^{-5})	1419 ± 83	1498 ± 77	NS
Glucose, mg/dl	83.7 ± 2.2	79.5 ± 1.0	NS
LDL-cholesterol, mg/dl	115.1 ± 8.6	101.8 ± 5.8	NS
HDL-cholesterol, mg/dl	39.9 ± 1.5	40.7 ± 1.8	NS
Triglycerides, mg/dl	93 (58)	87 (63)	NS
C-reactive protein, mg/dl	0.74 (0.86)	0.17 (0.77)	0.033

Continuous normal and non-normal variables are presented as mean ± SEM and median (25–75th percentile), respectively. SCI — spinal cord injury; PVR — peripheral vascular resistance; LDL — low density lipoprotein; HDL — high density lipoprotein.