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Oxidized low-density lipoprotein, matrix-metalloproteinase-8 and carotid atherosclerosis in spinal cord injured subjects

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ABSTRACT

Objective: Previous reports have indicated that subjects with chronic spinal cord injury (SCI) exhibit increased cardiovascular risk compared to able-bodied individuals. This study investigated the relationship between plasmatic oxidized low-density lipoprotein (OxLDL), matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) levels and vascular remodeling in SCI subjects and the role of physical activity in this regard.
Methods: We studied 42 men with chronic (≥2 years) SCI [18 sedentary (S–SCI) and 24 physically active (PA–SCI)] and 16 able-bodied men by clinical, anthropometric, laboratory, and carotid intima-media thickness (IMT) analysis. All enrolled subjects were normotensive, non-diabetics, non-smokers and normolipemic. Plasmatic OxLDL, MMP-2, MMP-8, MMP-9, TIMP-1 and TIMP-2 levels were determined by enzyme-linked immunosorbent assay.
Results: Carotid IMT, IMT/diameter ratio and OxLDL levels of PA–SCI and able-bodied subjects were statistically similar. Conversely, S–SCI subjects exhibited higher IMT, IMT/diameter ratio and OxLDL levels compared to PA–SCI ($p < 0.01$, $p < 0.001$ and $p = 0.01$, respectively) and able-bodied ($p < 0.001$ for all) individuals. Results of bivariate correlation analysis including all injured subjects showed that carotid IMT and IMT/diameter ratio only correlated with OxLDL, MMP-8 and MMP-8/TIMP-1 ratio. Further stepwise regression analysis adjusted for the presence or not of physical activity and age showed that OxLDL was associated with carotid IMT and IMT/diameter ratio, while MMP-8 was associated with IMT/diameter ratio in SCI individuals.
Conclusions: Plasmatic OxLDL and MMP-8 levels are associated with carotid atherosclerosis and there is an interaction among physical inactivity, atherosclerosis and OxLDL in SCI individuals.

1. Introduction

Cardiovascular diseases are commonly seen in subjects with spinal cord injury (SCI) and coronary heart disease is more prevalent in individuals with SCI than in the able-bodied population [1]. For long-term SCI, morbidity and mortality from cardiovascular causes now exceed those caused by renal and pulmonary conditions, the primary causes of mortality in previous decades [2].

Several reports have shown that subjects with SCI exhibit increased subclinical atherosclerosis in comparison with able-bodied individuals, independent of traditional cardiovascular risk factors [3–6]. In addition, other lines of evidence have indicated that regular physical activity is associated with decreased carotid intima-media thickness (IMT) in SCI individuals, independent of variation in hemodynamic, metabolic and inflammatory variables [7,8]. These data point toward chronic SCI as a potential condition for the study of the mechanisms underlying physical inactivity-induced atherogenesis.

The oxidatively modified form of low-density lipoprotein-cholesterol (OxLDL) as well as matrix metalloproteinases (MMPs)

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and tissue inhibitors of MMPs (TIMPs) are known to play an important role in atherogenesis [9,10]. In addition, circulating concentrations of OxLDL, MMPs and TIMPs have been also related to atherosclerosis development and progression in human beings [9–11]. Former studies revealed that regular physical activity is associated with decreased levels of OxLDL and MMPs in able-bodied subjects, which might contribute to explain the beneficial effects of exercise on cardiovascular risk [12–14]. Therefore, the present study investigated the carotid arteries of physically active (PA–SCI) and sedentary (S–SCI) men with SCI and able-bodied men and evaluated the impact of OxLDL, MMPs and TIMPs in this regard.

2. Materials and methods

2.1. Study population

A total of 42 [18 S–SCI (age = 32.3 ± 1.8 years; time of injury = 7.2 ± 1.1 years) and 24 PA–SCI (age = 30.1 ± 1.3 years; time of injury = 9.3 ± 0.9 years)] men with at least two years of SCI and 16 sedentary able-bodied men (age = 29.6 ± 1.3 years) were cross-sectionally evaluated. S–SCI subjects were enrolled from the hospital of the University of Campinas, while able-bodied individuals were recruited from employees and students of the same university. PA–SCI subjects comprised competitive athletes who were regularly performing wheelchair rugby ($n = 12$), basketball ($n = 11$) and handball ($n = 1$) for at least one year and were enrolled from the School of Physical Education of the University of Campinas. This latter group had been participating in physical activities in average 11.9 ± 1.4 h per week for 4.4 ± 0.6 years. Enrolled able-bodied men did not perform sports or recreational physical activity. Exclusion criteria for all groups included diabetes mellitus, systemic hypertension, hyperlipidemia [15], current or past smoking, known coronary artery, cardiac or pulmonary disease, cancer, regular medical therapy and clinical evidence of active infection. All enrolled SCI subjects presented no preserved motor function below the injury level.

2.2. Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of the University of Campinas. All participants read and signed informed consent.

2.3. Clinical, laboratory and hemodynamic data

Clinical data included information on the participants' age and injury duration. Stature of SCI individuals was taken using a stadiometer (WCS model, Cardiomed, Curitiba, Brazil) in supine position. Body mass index was calculated as body weight divided by height squared. Blood samples were obtained on 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, Kyoto, Japan). Two readings were averaged and if they differed by more than 5 mmHg, one additional measurement was performed and then averaged.

2.4. Oxidized LDL and 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 kits were used to evaluate the plasmatic concentrations of OxLDL (Mercodia AB, Uppsala, Sweden), and MMP-9, MMP-2, MMP-8, TIMP-1 and TIMP-2 concentrations (R&D Systems, Minneapolis, MN, USA).

2.5. Carotid ultrasonography studies

Carotid ultrasonography studies were performed by a skilled physician on each subject in the sitting position with a Vivid 3 Pro apparatus (General Electric, Milwaukee, WI, USA) equipped with a 10-MHz transducer [16,17]. The average from both right and left common carotid arteries measurements was used for analyses. In order to measure the carotid IMT, a region 2 cm proximal to the carotid bifurcation was identified, and the IMT of the far wall was evaluated as the distance between the lumen–intima interface and the media–adventitia interface. All measurements were made using an automatic border recognizer (Vivid 3 Pro IMT software analyzer) on still images obtained during the sonographic scanning. No plaques were visualized while measuring IMT. End-diastolic internal carotid diameters were obtained by continuous tracing of 3 cycles and averaged. Intraobserver and interobserver carotid IMT and diameter variabilities were <5%.

2.6. Statistical analysis

Results were analyzed using SPSS 15.0™. Continuous normal and non-normal variables are presented as mean ± standard error and median (25–75th percentile), respectively. Based on previous studies [3,8], a sample size of 15 individuals in each group was considered suitable for detecting significant differences in carotid IMT regarding values of alpha error = 0.01 and beta error = 0.9. However, we were able to extend data collection to 16, 18 and 24 individuals in the able-bodied, S–SCI and PA–SCI groups. The Kolmogorov–Smirnov test was used to test for normal distribution of the variables. Differences in continuous normal variables were evaluated by one-way ANOVA followed by Tukey test for pairwise comparisons, while differences in continuous non-normal variables were evaluated by Kruskal–Wallis test followed by Wilcoxon signed rank test for pairwise comparisons. 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 for non-normal data. Partial correlation analysis was used to evaluate the relationship between OxLDL and MMP-2 after adjustment for physical activity. Stepwise regression analysis was used to evaluate the independent predictors of carotid IMT and IMT/diameter. Variables that exhibited significant correlation at bivariate analysis were included as independent variables in regression analyses. A p-value <0.05 was considered significant.

3. Results

Clinical, hemodynamic and carotid features of enrolled subjects are presented in Table 1. Able-bodied subjects exhibited higher blood pressure levels than the SCI groups, higher average body mass index than the PA–SCI group and lower C-reactive protein levels than the S–SCI group. In addition, average carotid IMT and IMT/diameter values of able-bodied and PA–SCI subjects were similar. However, S–SCI subjects exhibited higher IMT and IMT/diameter ratio compared to PA–SCI ($p < 0.01$ and $p < 0.001$, respectively) and able-bodied ($p < 0.001$ for both) individuals. Plasmatic levels of OxLDL, MMPs and TIMPs are presented in Table 2. OxLDL levels of able-bodied and PA–SCI subjects were similar. Nevertheless, S–SCI individuals showed higher OxLDL levels than PA–SCI ($p = 0.01$) and able-bodied ($p < 0.001$) subjects. Al last, no differences in MMPs, TIMPs and MMP/TIMP ratios were detected among the studied groups.