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Differential levels of cathepsin B and L in serum between young and aged healthy people and their association with matrix metalloproteinase 2

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ABSTRACT

Objective: Most publications describe cathepsin B and L as tumor and metastasis factors. These proteases also play a very important role in aging process. The aim of this study was to evaluate the serum level of cathepsin B and L with aging and their association with matrix metalloproteinase 2 (MMP2), which was reported to associate with age-related diseases.
Methods: This research was conducted using blood samples provided by healthy people (n = 90, 63 men and 27 women). Subjects were subdivided into groups with respect to age: young (about 18–30 years old, n = 30), middle age (about 36–50 years old, n = 30), and aged (above 56 years old, n = 30). Altered serum level of cathepsin B, cathepsin L, and MMP2 with aging was studied by enzyme-linked immunosorbent assay (ELISA) and Western blotting using discriminative antibodies specific for each factor.
Results: ELISA and Western blotting revealed that the serum level of cathepsin L and MMP2, but not cathepsin B significantly decreased in aged group compared with young group. Cathepsin L positively correlates with MMP2 among the whole healthy people (r^2 = 0.869, $p < 0.0001$).
Conclusion: The serum level of cathepsin L decreased with age, while cathepsin B remained no significant difference between young and aged individuals. In addition, cathepsin L positively correlates with MMP2.
Practice: The cathepsin L may be used as a monitoring index in age-related diseases.
Implications: In addition to cathepsin B, cathepsin L may be also involved in the aging process.

1. Introduction

Oxidative stress and low-grade inflammation are the hallmarks of the aging process and are even more enhanced in many age-related degenerative diseases. Mitochondrial dysfunction and oxidative stress can provoke and potentiate inflammatory responses (Salminen, Ojala, Kaarniranta, & Kauppinen, 2012).

Lysosomes are the key degradative compartments of the cell. Lysosomal cathepsins, which are enclosed in the lysosomes, help to maintain the homeostasis of the cell's metabolism by participating in the degradation of heterophagic and autophagic material (Repnik, Stoka, Turk, & Turk, 2012). During aging, lipofuscin accumulates in lysosomes of post-mitotic cells. These insoluble aggregates may physically cause lysosomal damage. Lysosomes are iron-rich organelles and thus sensitive to reactive oxygen species-mediated oxidation of proteins and lipids (Kurz, Eaton, & Brunk, 2011), which can also induce lysosomal membrane rupture. The membrane damage can lead to the release of cathepsin B, which is known to stimulate inflammasomes. For instance, cholesterol crystals activate nod-like receptor protein 3 (NLRP3) inflammasomes via cathepsin B release in human macrophages (Rajamäki et al., 2010; Salminen et al., 2012). In another study, cholesterol crystals could induce peritonitis which did not occur in mice deficient in NLRP3, cathepsin B and L, or IL-1b (Duewell et al., 2010). Wyczałkowska-Tomasik and Pączek (2012) observed that the activity of cathepsin B and L together in the serum significantly increases with age. But what is the difference expression of cathepsin B and L in aging process remains to be further studied. Age-associated central arterial wall stiffness is linked to extracellular matrix (ECM) remodeling, including fibrosis and vascular

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calcification. Matrix metalloproteinases (MMP) play an important role in ECM remodeling (Shen et al., 2015); some studies also showed that activated MMP2 related to aging (Jiang et al., 2012; Kvetnaia, Lin'kova, Sedov, Mursalov, & Kozlov, 2013; Wang et al., 2005). Recently, cathepsins have also been demonstrated to play an important role in ECM remodeling and are implicated in the development and progression of age-related vascular disease (Cheng et al., 2006, 2008).

In this study, we detected the serum level of cathepsin B, cathepsin L, and MMP2, respectively, in different age groups and analyzed the relationship between cathepsins and MMP2.

2. Materials and methods

In a standardized interview, participants were asked about height and weight, history of hypertension, stroke, osteoporosis, hormones treatment or cardiovascular disease, and smoking. Furthermore, all participants measured their blood pressure at home at six different time points during the day (Omron MX3; Omron, Mannheim, Germany). Fasting glucose, C-reactive protein (CRP), fasting triglycerides, and fasting cholesterol were determined. Body mass index (BMI) was calculated as weight divided by the square of height. Hypertension was defined as an average systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg or self-reported use of blood pressure-lowering drugs.

This research was conducted on blood samples provided by healthy individuals. The samples were divided by age into samples from individuals who were: young (about 20 years old, mean 24.6 years, age range 18–28 years, n = 30, 21 men and 9 women), middle age (about 40 years old, mean 41 years, age range 36–50 years, n = 30, 24 men and 6 women), and elderly (about 60 years old, mean 65 years, age range 56–78 years, n = 30, 18 men and 12 women).

Each person gave informed consent to the procedure. The protocol of the study was approved by the ethics committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China).

2.1. ELISA

Serum aliquots were used to measure human cathepsin L, cathepsin B, and MMP2 levels by enzyme-linked immunosorbent assay (ELISA) according to a protocol provided by the manufacturer (Bender MedSystems, Burlingame, CA). Briefly, 96-well ELISA plates were coated with antibody overnight. Plates were blocked with 0.5% bovine serum albumin in 1× phosphate buffered saline with 0.05% Tween-20. Next, 100 μl of diluted human serum (2:1) samples and 50 μl of biotin-conjugated antibodies were added to each well, followed by overnight incubation. Plates were washed before adding 100 μl of streptavidin–horseradish peroxidase. Plates were developed using OPD (Sigma) and a standard curve was created from each plate as recommended. A representative of three independent measurements was presented. All values were adjusted to the total protein level.

2.2. Western blot analysis

Western analysis was performed as previously described (Wang et al., 2014). Proteins were extracted from serum samples using CelLytic MT Cell Lysis Reagent (Sigma) and separated on 10% sodium dodecyl sulfate-polyacrylamide gels. Proteins were probed with the following antibodies: anti cathepsin L (1:1000, Abcam), cathepsin B (1:1000, Abcam), MMP2 (1:1000, BD Biosciences), β-actin (1:1000, Sigma), were used as an internal control. The blots were scanned with an Odyssey imager (LI-COR Biosciences) and band intensity was determined with Quantity One System (Bio-Rad).

2.3. Statistical methods

All data are expressed in terms of mean ± standard deviation. For the population characteristics, between-group differences were analyzed with t test for means, Mann–Whitney U test for nonparametric data, and χ^2 test for proportions. A non-parametric Spearman's correlation test was applied to examine the correlation of cathepsins with CRP, MMP2, sex and age among the whole healthy people. Linear regression analysis was applied to examine the linear correlation of cathepsin L and MMP2. The results were considered statistically significant when $p < 0.05$. The software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, United States) was used for statistical analysis.

3. Results

3.1. Clinical data

As noted in Table 1, the entire cohort (n = 90), 63 men and 27 women, was included in this study. They were divided into three groups by age: young group (about 20 years old, mean 24.6 years, n = 30), middle-age group (mean 41 years, n = 30), and aged group (mean 65.0 years, n = 30).

There was no significant difference between young and aged groups in clinical characteristics such as BMI, fasting plasma glucose, smoking habit, serum cholesterol, triglycerides, systolic blood pressure, and diastolic blood pressure. But the serum level of CRP significantly increased in aged group compared with young group (p = 0.02).

3.2. The altered serum level of cathepsin B, cathepsin L, and MMP2 with age

The serum level of cathepsin B is equal for young group 0.050 ± 0.031 ng/mL, for middle-age group 0.053 ± 0.32 ng/mL, and for aged group 0.046 ± 0.014 ng/mL, there is no significant difference in the three groups. The serum level of cathepsin L and MMP2 is 0.96 ± 0.48 ng/mL and 760.0 ± 360.0 ng/mL, respectively, for young group, 0.92 ± 0.53 ng/mL and 730.0 ± 440.0 ng/mL, respectively, for middle-age group, and 0.77 ± 0.18 ng/mL and 570.0 ± 130.0 ng/mL, respectively, for aged group. The serum level of cathepsin L and MMP2 was significantly decreased in aged group compared to young group (p = 0.04 for cathepsin L and p = 0.01 for MMP2), but not in the middle-age group. (Table 2). Western blot analysis revealed that cathepsin L and MMP2 significantly decreased in aged serum samples compared to young serum samples, while cathepsin B had no significant difference between the two groups (Fig. 1).

Table 1
Clinic characteristics of the study cohort.

	Young (n = 30)	Middle-age (n = 30)	Aged (n = 30)	p Value
Age (years)	24.6 ± 2.7	41.4 ± 3.8	64.5 ± 6.0	–
BMI (kg/m^2)	20.7 ± 1.2	20.0 ± 2.6	21.0 ± 2.1	0.53
Smoking habit (%)	7 (26%)	8 (28%)	9 (30%)	0.06
CRP (mg/L)	0.6 ± 1.2	0.7 ± 1.9	1.9 ± 2.5	0.02
FPG (mmol/L)	5.6 ± 1.5	5.8 ± 1.7	6.0 ± 1.4	0.07
TC (mmol/L)	5.2 ± 0.9	5.3 ± 0.8	5.4 ± 0.7	0.06
TG (mmol/L)	1.5 ± 0.9	1.6 ± 0.7	1.7 ± 0.8	0.08
SBP (mmHg)	135.0 ± 16.0	137.0 ± 18.0	139.0 ± 15.0	0.42
DBP (mmHg)	85.0 ± 8.0	86.0 ± 8.7	88.0 ± 7.7	0.08

BMI: body mass index; CRP: C-reactive protein; FPG: fasting plasma glucose; TC: fasting serum cholesterol; TG: fasting triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure; data are expressed as mean ± standard deviation or percentage. p Value indicated aged group compared with young group.