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Observational and mechanistic links between C-reactive protein and blood pressure in elderly women

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ABSTRACT

It is hypothesized that chronic systemic inflammation contributes to the age-related decline in cardiovascular function. The aim of the present study was to combine an assessment of the relationship between the serum level of C-reactive protein (CRP) and systolic and diastolic blood pressure in 108 elderly women (65 and 70 years) with an *in-vitro* exploration of the effects of CRP on the proliferative and angiogenic potential of endothelial cells exposed to serum in elderly women. Based on the median CRP level in our population, LowCRP (CRP < 1.3 mg/L) and HighCRP (>1.3 mg/L) groups were identified. Body mass index, waist circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in the HighCRP group than in the LowCRP group ($p < 0.05$). The influence of CRP on SBP and DBP remained significant after adjustments for BMI and use of antihypertensive medication ($p < 0.05$). When adjusting for waist circumference the observed influence of CRP on SPB was attenuated ($p = 0.062$). We next evaluated the ability to form capillary tubes (angiogenesis assay) and the proliferation rate of endothelial cells exposed to the sera of elderly women. Increased serum CRP levels were associated with an increased doubling time of endothelial cells ($R^2 = 0.39$; $p < 0.05$) and decreased capillary tube length ($R^2 = 0.30$; $p < 0.05$), indicating a reduction in the proliferation rate of endothelial cells and angiogenic potential. In conclusion, chronic inflammation influences blood pressure in elderly women and compromises endothelial cell function, thus contributing to the age-related decline in vascular health.

1. Introduction

It is hypothesized that chronic systemic inflammation is associated to the age-related decline in cardiovascular function [1,2]. Elevations in the level of the circulating acute phase C-reactive protein (CRP), a biomarker for systemic inflammatory burden, is associated with increased risk of cardiovascular disease [3,4], and has out of a panel of 9 inflammatory biomarkers been identified as a significant predictor of developing hypertension [5]. An elevation of CRP level has recently been shown in subjects with prehypertension (blood pressure ranging from 120 to 139 mm Hg) [6] and it has been shown that hypertension was significantly associated with higher CRP levels in both men and women [7]. However, ethnic differences were evident and no association among the Hispanic population was observed. Furthermore, a number of reports based on different ethnic populations have suggested that elevated CRP levels may precede the onset of hypertension [8,9]. It has also been shown that having a high CRP level together with abdominal obesity was associated with a significantly increased risk of new-onset hypertension [10].

Endothelial cell dysfunction has been suggested to play an integral part in age-related development of hypertension [11]. *In-vitro* studies have shown that exposure to exogenous commercial CRP causes endothelial cell dysfunction [12]. Although previous studies suggest a link between CRP level and blood pressure, few have been reported on elderly women populations specifically. Furthermore, no previous study has investigated potential adverse effects of CRP on endothelial cell proliferative rate and angiogenic potential by exposing endothelial cells to sera from elderly individuals. Such an approach would enable the study of the influence of serum CRP level on the endothelial function using a model that incorporates important factors present in the physiological environment of endothelial cells.

The aim of the present study was to combine an assessment of the influence of serum CRP level on systolic and diastolic blood pressures in a sample of elderly women with an exploration of the influence of CRP in the serum of elderly women on the *in-vitro* proliferation rate and angiogenic potential of endothelial cells.

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2. Materials and methods

2.1. Subjects

One hundred and eight women aged between 65 and 70 years were recruited through advertisement in a local newspaper. A medical history and electrocardiograms were assessed by a physician. Subjects included in the study were free of diabetes mellitus, had no disability in regard to mobility, were non-smokers and were not using anti-inflammatory medication. For the in-vitro model, we used serum from 15 subjects, which were selected out of the 108 women to include a wide range of CRP values in order to explore potential influence of different CRP levels on endothelial cell functions. After verbal and written information, all participants gave their written informed consent, and ethical approval was obtained from the regional ethical review board in Uppsala.

2.2. Anthropometrics, blood pressure and physical fitness

Body weight (kg) and height (cm) were measured using a digital scale and a portable stadiometer. BMI was calculated by as body weight (kg) divided by the square of height (m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined in the right arm using a manual mercury sphygmomanometer after a 5-min rest in the supine position. Participants performed a submaximal exercise test to assess physical fitness levels. They exercised on a cycle ergometer (model 874 E; Monark, Varberg, Sweden) for 6 min at a constant workload at 50 rpm. Heart rate was measured throughout the test, and the average heart rate during the last two minutes was used to predict subjects VO_2max (ml kg min^{-1}) [13].

2.3. High-sensitivity C-reactive protein

Blood samples were obtained between 7.00 and 9:00 am, after an overnight fast. The participants were asked to avoid alcohol and not to engage in any strenuous physical activity 24 h before the blood sample. Blood was collected by venipuncture from an antecubital vein, centrifuged at 4000 rpm for 10 min, aliquoted and stored in −80 °C. CRP level was measured using a high-sensitivity C-reactive protein (Hs-CRP) kit by a fully automated immunoturbidimetric assay (Advia 1800, Chemistry System, Siemens, Germany).

2.4. In vitro *model to study the effect of serum CRP level from elderly on endothelial cell proliferation*

In order to explore the influence of serum CRP level on *in-vitro* endothelial cell function, human umbilical vein endothelial cells (HUVEC) were incubated in sera from 15 elderly women. HUVEC were purchased from Promo Cell, cultured in endothelial cell basal medium-2 (EGM-2) and used for experiments at passage 4. HUVEC were cultured in 24-well plates at an initial seeding density of 5×10^3 cells per well. Cells were incubated in medium with 5% serum obtained from each subject. HUVEC were grown under standard incubation conditions at 37 °C in 5% CO_2 for 96 h. Doubling time (DT) was calculated using the formula: DT = 96/(ln(y/x)/ln2), where x is the number of cells counted on day 0 and y the number of cells counted at day 4; 96 is representative of the number of hours HUVEC spent in incubation [14].

2.5. Endothelial cell proliferation in CRP-treated HUVEC and control HUVEC

To further confirm the influence of serum CRP level from elderly on endothelial cell proliferation, HUVEC were treated with commercial CRP (C1617, sigma) on a 24-well plate at a seeding density of 5×10^3 cells per well. The cells were cultured in EGM-2 without the addition of CRP (control HUVEC) and with the addition of CRP (15 μg/mL) (CRP- HUVEC), a concentration previously used for the study of the effects of CRP on endothelial cell function [15]. A total of 3 experiments in duplicate were performed.

2.6. In vitro *model to study the effect of serum CRP level from elderly on the angiogenic potential of endothelial cells*

In-vitro matrigel angiogenesis assay was used to assess the effects of serum CRP level on the angiogenic potential of HUVEC. Twenty-four well plates were coated with growth factor-reduced matrigel (BD Biosciences, Bedford, MA, USA) and HUVEC (1.2×10^5/well) were supplemented with 5% sera from 15 elderly subject for an incubation period of 18 h. Calcein AM fluorescent dye was used to visualize capillary tubes. Capillary tube formation was assessed under a phase contrast microscope coupled to a Carl Zeiss camera (AxioCam ICC 1) and quantified using Sigma Scan Pro 5 software. The total length of the capillary tubes is assessed in a culture dish area of 100 mm^2. Capillary tube formation is expressed as mm tube length/mm^2 of culture dish area.

2.7. Statistical analysis

All data was tested for normality using Shapiro-Wilk normality test and skewed data was log transformed. The variables age, height, weight, BMI, waist circumference, systolic pressure, diastolic pressure, physical fitness are presented as mean ± SD. Due to skewness CRP was presented as geometric mean. In accordance with previous reports [10] two groups were formed based on CRP median value (1.3 mg/L): LowCRP (CRP < 1.3 mg/L) and HighCRP (>1.3 mg/L). First, between-group comparisons were conducted using independent samples *t*-tests. Second, in order to control for confounding effects of adiposity on the proposed influence of CRP level on blood pressure, analysis of covariance (ANCOVA) was conducted with either BMI or waist circumference included as a continuous covariate. Use of medication related to cardiovascular health (yes, no) was additionally included in the model. Linear regression was used to examine the effects of serum CRP level on endothelial cell proliferation and angiogenic potential. P values less than 0.05 were considered as statistical significant. All statistical analyses were performed using SPSS software (version 20.0).

3. Results

The age, BMI, waist circumference, SBP, DBP, CRP level and physical fitness of all women together and stratified by CRP level are presented in Table 1. Thirty-three women (30.5% of the population) were taken antihypertensive medication. Both BMI and waist circumference were significantly higher in the HighCRP group compared to the LowCRP group ($p < 0.01$). A non-significant ($p = 0.059$) difference in physical fitness was observed between CRP groups. A simple between-group comparison showed that both SBP and DBP were significantly higher in the HighCRP group compared to the LowCRP group ($p < 0.01$). These between-group differences in SBP and DBP remained significant after adjustments for use of antihypertensive medication and BMI were made (ANCOVA) ($p < 0.05$). However, when adjusting for waist circumference instead of BMI the observed influence on SPB was attenuated ($p = 0.062$).