Atherosclerosis 211 (2010) 579–583

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Serum levels of total *p*-cresylsulphate are associated with angiographic coronary atherosclerosis severity in stable angina patients with early stage of renal failure

Chao-Ping Wang[a,e], Li-Fen Lu[b], Teng-Hung Yu[a], Wei-Chin Hung[a], Cheng-An Chiu[a], Fu-Mei Chung[a], Lee-Ren Yeh[d], Han-Jung Chen[c], Yau-Jiunn Lee[f], Jer-Yiing Houng[e,*]

[a] *Division of Cardiology, Department of Internal Medicine, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan*
[b] *Division of Cardiac Surgery, Department of Surgery, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan*
[c] *Division of Neurology, Department of Surgery, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan*
[d] *Department of Radiology, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan*
[e] *Department of Medical Nutrition, Institute of Biotechnology and Chemical Engineering and I-Shou University, Kaohsiung 82445, Taiwan*
[f] *Lee's Endocrinologic Clinic, Pingtung 90000, Taiwan*

ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 25 March 2010
Accepted 25 March 2010
Available online 4 April 2010

Keywords:
Total *p*-cresylsulphate
Stable angina
Modified Gensini score

ABSTRACT

Objective: *p*-Cresylsulphate (PCS), a protein-bound uraemic retention solute, is known to cause endothelial dysfunction and possibly plays a role in coronary atherosclerosis. We aimed to investigate the relationship of total PCS with traditional biomarkers associated with chronic coronary atherosclerosis. In addition, the relationship between serum total PCS levels and the severity of coronary artery stenosis was also explored.

Methods and results: Serum total PCS concentrations were measured by using the Ultra Performance LC System in 202 consecutive stable angina patients, and their associations with angiographic indexes of the number of diseased vessels and modified Gensini score were estimated. Patients with significant coronary artery stenosis have higher median serum total PCS levels than patients with normal coronary arteries. Statistically significant associations were observed between the serum total PCS levels and the number of diseased vessels ($\beta = 0.261$, $p = 0.0002$), and modified Gensini score ($\beta = 0.171$, $p = 0.016$). Using multivariate analysis, serum total PCS level was independently associated with the presence and severity of CAD.

Conclusions: This study indicates that serum total PCS levels are significantly higher in the presence of CAD and are correlated with the severity of the disease, which suggest that increased serum total PCS may be involved in the pathogenesis of coronary atherosclerosis.

1. Introduction

High cardiovascular disease (CVD) prevalence and mortality in chronic kidney disease (CKD) patients have been both a conundrum and bothersome to clinicians for a long time. Both chronic kidney disease (CKD) and coronary artery disease (CAD) share several common pathogenetic pathways. Mounting data point to the lethal synergy between CKD and CVD [1]. CKD with renal toxins retention have been implicated in the pathogenesis of accelerated atherosclerosis as well [2]. Although the significant relationship between CKD and renal toxins has been studied and reviewed [3], CKD is simply a global renal function status, not like renal toxins which provides a meaningful and predominant direct reaction causing vascular injury [4].

p-Cresol (4-methylphenol, molecular weight 108.1 Da) is a small molecule derived from ingested phenylalanine and plant phenols and in humans exists predominantly as the conjugate *p*-cresylsulphate (PCS), which is a protein-bound rich substance. In a new post hoc analysis evidence has been presented that higher free form *p*-cresol levels in dialysis patients are associated with cardiovascular events suggesting it may be a novel cardiovascular risk factor [5]. *p*-Cresol and PCS have been implicated as an important contributor to inhibition of phagocyte reactive species production and endothelial dysfunction by enhancing baseline leukocyte activity [6,7]. PCS binds strongly to protein and is thus poorly cleared with conventional hemodialysis [8]. PCS also has a pro-inflammatory effect and produces free radicals as evaluated by the increased oxidative burst activity of leucocytes at baseline, therefore, PCS may contribute to the propensity to vascular damage in CKD patients [7]. Although, these evidences of biological toxicity of PCS support its role as a possible cardiovascular risk factor, the researchers did not provide data on the association between total PCS levels and the severity or extent of CAD. In

* Corresponding author. Tel.: +886 7 6577711x3415; fax: +886 7 6578945.
E-mail addresses: jyhoung@isu.edu.tw, gene6623@yahoo.com.tw, ed100232@edah.org.tw (J.-Y. Houng).

0021-9150/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2010.03.036

the present study, which is aimed at investigating this hypothesis, we examined whether total PCS levels might be associated with selected atherosclerotic inflammatory cytokines and traditional cardiovascular risk markers in a cohort of stable angina patients. We further assessed serum total PCS levels, angiographic indexes of the number of diseased vessels and modified Gensini score in this population.

2. Methods

2.1. Participants

The study population consisted of 202 consecutive consenting patients who underwent first-time angiography with clinical diagnosis as stable angina from June 2006 to June 2008 at the Cardiovascular Clinic of E-Da Hospital. Stable angina pectoris was defined as effort-related chest pain without evidence of recent deterioration or rest pain in the previous 6 months. The patients with histories of concomitant inflammatory diseases (such as infection, sepsis, malignancy, liver disease, and collagen disease), steroid use or surgery within 1 month prior to admission on the basis of the interview, physical examination, biochemistry lab data and urinalysis were excluded from the study. The study protocol was approved by the Human Research Ethics committee of the hospital, and informed consent was obtained from each patient.

2.2. Laboratory measurements

Peripheral blood samples were taken from an antecubital vein after admission to the hospital. Blood for total PCS determination was drawn, centrifuged, and stored at −80 °C for subsequent assay. Before coronary angiography, complete blood counts and serum creatinine and serum lipid profiles were determined in all patients. Plasma triglycerides, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), uric acid, albumin, creatinine, and glucose were measured by standard commercial methods on a parallel, multi-channel analyzer (Hitachi 7170A, Tokyo, Japan) as our previous reports [9].

For determination of total PCS serum levels, samples were deproteinized by the addition of 3 parts methanol to 1 part serum. The total PCS was measured in serum ultrafiltrates obtained using a UPLC assay. A UPLC assay, using detection at the 280 nm of the PDA detector, was performed at room temperature on an ACQULITY UPLC® BEH phenyl column of 2.1 mm × 100 mm. The buffer flow was 0.4 mL/min using 10 mM $NH_4H_2PO_4$ (pH = 4.0) (A) and 100% acetonitrile (B) with a gradient from 82.5%A/17.5%B to 55%A/45%B, over 9 min. Under these conditions, *p*-cresol sulphate was detected at the 260 nm and appeared at 1.7 min. There were standard curves from total PCS at 0.5, 1, 2.5, 5, and 10 mg/L; processed like-serum samples had average r^2 values of 0.999 ± 0.001. Quantitative results were obtained and calculated as concentrations (mg/L). The method detection limit of this assay was 1 mg/L [10]. The concentration of plasma visfatin was determined using a commercial enzyme immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). Adiponectin, leptin and E-selectin levels were determined by commercial solid phase enzyme linked immunosorbent assay kits (B-Bridge International, Sunnyvale, CA and Phoenix Pharmaceuticals, Belmont, CA and R & D Systems, Inc., USA, respectively). The intra-assay coefficients of variation of assay were 2.4–2.7% for visfatin, 3.2–7.3% for adiponectin, 3.2–6.9% for leptin and 5.2–6.6% for E-selectin. Samples were measured in duplicate in a single experiment. To describe the true CCr as closely as possible, the estimated CCr used a primary estimate of renal function. This was serum creatinine, estimated CCr calculated by the modification of estimated GFR calculated with the MDRD extended version as in our previous reports [11].

2.3. Angiographic definitions

Coronary angiograms were obtained according to standard techniques, and the severity of stenosis was assessed using quantitative coronary angiography. Angiograms and quantitative coronary angiographic analysis were evaluated by at least 2 experienced interventional cardiologists blinded to clinical information and serologic parameters and were scored according to 2 scoring systems: (1) the possible scores of this index ranged from 0 to 3 diseased vessels. The criterion for 1-, 2-, or 3-vessel disease was a >75% reduction in the internal diameter. The diameter of stenosis of the left main coronary artery could not exceed 50%. (2) In the modified Gensini scoring system, weights are assigned to each coronary segment depending on vessel size and importance, ranging from 0.5 to 5.0; segments serving larger regions of the myocardium are more heavily weighted. The narrowing of the coronary artery lumen is rated 2 for 0–25% stenosis, 4 for 26–50%, 8 for 51–75%, 16 for 76–90%, 32 for 91–99%, and 64 for 100%. The modified Gensini index is the sum of the total weights for each segment [12,13].

2.4. Statistical analysis

Data normality was analyzed using the Kolmogorov–Smirnov test. Continuous, normally distributed variables are presented as mean ± SD, and non-normally distributed variables as median (interquartile range). Statistical differences in variables were compared using unpaired Student's *t*-tests for normally distributed variables. Categorical variables were recorded as frequencies and/or percentages, and inter-group comparisons were analyzed by the chi-square test. Since the distributions of serum total PCS, visfatin, leptin, E-selectin, adiponectin and triglyceride were skewed; logarithmically transformed values were used for statistical analysis. Simple and multiple linear stepwise regression analyses were used to examine the correlations and independence between serum total PCS and the values of other parameters. Using multiple logistic regression, these variables were assessed for independent associations with the presence of CAD. Association of total PCS and severity of CAD were analyzed by logistic regression and used the non-vessel disease group as the referent group. For stratified analysis, we also calculated the multivariable-adjusted odd ratio (OR) associated with a doubling in severity of coronary artery disease. Multivariate adjusted ORs are presented with 95% confidence interval (CI). Statistical significance was accepted if $p < 0.05$. All of the statistical analyses were performed using SAS statistical software, version 8.2 (SAS Institute Inc.; Cary, NC).

3. Results

In the present study, we found that median serum total PCS levels in patients with CAD were significantly higher than levels in 39 randomly selected healthy subjects without known CAD, hypertension, diabetes or major systemic diseases (1.7 mg/L (interquartile range 1.0–6.3) vs. 1.0 mg/L (interquartile range 1.0–2.4), $p = 0.008$). Simple linear regression analysis revealed that total PCS was positively associated with age, blood urea nitrogen, creatinine, E-selectin and adiponectin. In addition, levels of hemoglobin, hematocrit, albumin and estimated GFR were negatively associated with levels of total PCS, while in multiple stepwise regression analysis, serum total PCS level was positively associated with fasting glucose, creatinine and negatively associated with albumin (Table 1).

We further assessed the relationship of the total PCS serum levels with CAD. There was no statistically significant difference in serum total PCS levels between men and women. Patient groups with and without traditional cardiovascular risk factors, did not have significantly different serum total PCS levels with the