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Coronary heart disease risk equivalence in diabetes and arterial diseases characterized by endothelial function and endothelial progenitor cell ☆

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ABSTRACT

Aims: Peripheral Arterial Disease (PAD), Carotid Artery Disease (CAD), and Type 2 Diabetes Mellitus (DM) were considered as "Coronary Heart Disease (CHD) risk equivalents". Vascular endothelial dysfunction was recognized as an early event in the development of atherosclerosis. Involved in neovasculogenesis and maintenance of vascular homeostasis, endothelial progenitor cell (EPC) has been considered as a biological marker of cardiovascular disease. The purpose of this study was to assess the CHD risk equivalents concept by investigating the endothelial function and circulating EPC number in patients with CHD, PAD, CAD and T2DM.
Methods: There were four groups in the study: CHD (n = 19), AD [PAD and CAD (n = 17)], DM (n = 21) and healthy controls (HC, n = 20). PAD and CAD were assessed by ultrasonography. Coronal artery angiography was used to identify CHD. The diagnosis of T2DM was based on oral glucose tolerance test and medical history. Vascular endothelial function was assessed by flow-mediated brachial artery dilatation (FMD). Circulating EPC was quantified by flow cytometry.
Results: The circulating EPC numbers in four groups were CHD, 973 ± 96; AD, 1048 ± 97; T2DM, 1210 ±125; HC, 1649 ± 112 cells/ml. There were no significant differences in circulating EPC numbers between CHD and AD groups ($P > 0.05$). Compared with CHD or AD group, T2DM group was associated with a slight increase in circulating EPC numbers ($P < 0.05$). The results of FMD were almost similar to the circulating EPC numbers(CHD, 4.06 ± 0.54; AD, 3.90 ± 0.48; DM, 3.85 ± 0.57; HC, 5.52 ± 0.67%)except that there was no significant difference among the CHD, AD and T2DM groups ($P > 0.05$). Age, glycosylated hemoglobin, low density lipoprotein cholesterol, systolic blood pressure, body mass index (BMI) and medical history were the independent risk factors of circulating EPC number in all the patients ($P < 0.05$). Age, total cholesterol, BMI and medical history were the independent risk factors of FMD in all of the patients ($P < 0.05$).
Conclusions: The results of this study supported the equivalents hypothesis and revealed that "CHD risk equivalents" were characterized by the consistent physiological changes of blood vessels in angiogenesis, repairing ability and endothelial function.

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1. Introduction

The coronary heart disease (CHD) risk equivalents refer to people without established CHD who will have an absolute, 10-year risk of developing major coronary events equal to that of persons with CHD. Peripheral arterial disease (PAD), carotid artery disease (CAD) and type 2 Diabetes (T2DM) have been regarded as CHD risk equivalents (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). It is important to identify those with CHD risk equivalents, as they belong to a high-risk category where primary prevention should be attempted. During the recent years, people with CHD risk equivalents have been investigated, however, the results were contradictory. Some studies confirmed the conclusion (Malmberg et al., 2000; Mukamal et al., 2001; Yusuf et al., 2000) and some studies reported opposite results (Cho, Rimm, Stampfer, Willett, & Hu, 2002; Lee, Folsom, Pankow, Brancati, & Atherosclerosis Risk in Communities (ARIC) Study Investigators, 2004; Lotufo et al., 2001; Vaccaro et al., 2004). In a retrospective cohort study, diabetic patients with no previous cardiovascular disease have the same long-term morbidity and mortality as nondiabetic patients with established cardiovascular disease after hospitalization for unstable coronary artery disease (Malmberg et al., 2000). Meanwhile, in a prospective cohort study, the magnitude of excess risk

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conferred by diabetes is similar to that conferred by a history of CHD For all-cause mortality; a history of CHD is a more potent predictor of death for mortality from CHD (Lotufo et al., 2001). Besides, no comparisons were made among the four groups (CHD, PAD, CAD and T2DM) in the same epidemiologic data. Furthermore, most studies focused on the epidemiologic data, and few on the pathophysiological processes.

The aim of this study was to reveal the "risk equivalents" meaning from the perspective of angiogenesis, repairing ability and endothelial function. The bone marrow-derived endothelial progenitor cells (EPCs) circulate in the blood and play an important role in the formation of new blood vessels as well as contribute to vascular homeostasis (Kawamoto et al., 2001; Rafii & Lyden, 2003). EPCs are obviously essential for these angiogenic and atherogenic processes. Recently, circulating EPC has been considered as a novel biomarker of both the ability of vascular repairing and the degree of vascular injury (Rosenzweig, 2005). Endothelial dysfunction is one of the earliest hallmarks of vascular abnormality (Verma, Buchanan, & Anderson, 2003). It appears to precede the clinical manifestations of many cardiovascular disorders, atherosclerosis for example, where abnormal vasoconstriction can be observed at the future site of plaque development (Abraham & Dashwood, 2008). As a noninvasive assessment, ultrasound measurement of brachial artery reactivity has been generally regarded as the gold standard for evaluation of endothelial systolic and diastolic function (Cohn, Quyyumi, Hollenberg, & Jamerson, 2004).

With this background, vascular endothelial function and the circulating EPCs number were investigated in normal control subjects and in patients with CHD, PAD, CAD and T2DM in our present study.

2. Subjects and methods

2.1. Study subjects

From May 2012 to November 2012, patients were recruited from the outpatients and inpatients in Wuhan Union Hospital. Control subjects were randomly chosen from the community residents who took health examinations in the hospital. The study was approved by the local ethics committee and informed consent was obtained from all subjects.

All subjects took a standardized evaluation including physical examination, laboratory tests, clinical diagnosis, and questionnaire on illness and medical treatment history. It was considered as positive if statins, ACE inhibitors and insulin had been continuously used in the last month before the test. Moreover, they underwent coronary artery angiography, ultrasonography and 75-g oral glucose tolerance test (OGTT). Predefined exclusion criteria were subject's refusal, more than seventy years of age or less than thirty years, acute illness or infection, neoplasm, recent surgery or vascular intervention, hemodialysis, immunosuppression and immunological diseases. During the examination, female subjects were not in the menstrual phase in order to avoid the effects on circulating EPC number.

The diagnosis of CHD was based on clinical history of myocardial infarction or angina pectoris or ischemic ECG changes. These were confirmed by coronary angiography. CAD was defined as mean carotid intima–media thickness (CIMT) > 1.00 mm and/or the presence of a plaque at any carotid location. PAD was defined as ankle brachial index ABI < 0.90 and/or the typical symptom of intermittent claudication or ischemic rest pain. These were confirmed by lower extremity arterial ultrasound. As patients with PAD and CAD were difficult to be distinguished clinically, we combined them as group AD. The diagnosis of T2DM was based on the results of OGTT and medical treatment history. Subjects in control group were recruited according to the following criteria: no past history of diabetes or cardiovascular diseases, normal glucose tolerance in OGTT, normal coronary angiography and ultrasonography. Finally, subjects in the study were divided into 19 patients with CHD (CHD group), 17 patients with CAD or PAD or both (AD group), 21 patients with T2DM (DM group) and 20 controls (HC group). It should be pointed out that patients with more than one type of disease (CHD, AD or T2DM) were excluded in order to avoid any interfering effects on endothelial function and EPCs number.

2.2. Ultrasound assessment of the brachial artery

The vascular studies of the brachial artery were performed noninvasively as previously described (Liao et al., 2010). High-resolution ultrasound (13 MHz linear array transducer; SSD prosound α10 color Doppler ultrasonograph, Aloka) was used to measure arterial diameter changes in response to both reactive hyperemia (an endothelium-dependent stimulus to vasodilation) and glyceryl trinitrate (GNT, an endothelium-independent vasodilator). The assessments were performed by the same operator who was well-trained and unaware of the clinical status of the patients. The brachial artery was scanned longitudinally above the antecubital fossa, with an inflatable cuff around the forearm. A baseline image was recorded and then the cuff was inflated to 300 mmHg for 5 min. After cuff deflation the artery was scanned continuously for 90 s. 15 min was allowed for vessel recovery. Subjects were then administered sublingual GNT (5 mg). The last scan was performed 4–5 min later. The electrocardiogram was monitored continuously. Vessel diameter was measured at end diastole, which coincided with the R-wave on the electrocardiogram. The mean diameter was measured at a fixed distance from an anatomical marker and calculated by four cardiac cycles. Flow-mediated dilation (FMD) was defined as the maximal change in dilatation from baseline during the 90 s after cuff deflation and was expressed as a percentage of the baseline diameter. GNT-mediated dilation (GMD) was defined as the maximal change in dilatation from baseline during the period 4–5 min after sublingual GNT and was expressed as a percentage of the baseline diameter.

2.3. Quantification of circulating EPC by flow cytometry

The number of circulating EPC was determined by the surface expression of $CD45^{low}/CD34^{+}/VEGFR2^{+}$ and assessed by flow cytometry as previously described (Chen et al., 2010). The assessments were executed in all subjects by the same well-trained operator who was unaware of the patients' clinical status. A total of 12-milliliter (ml) of peripheral venous blood in sodium heparin was collected from each subject. 5-mL was used for enumeration of circulating EPC number. Another 2-mL was used for identifying the absolute count of lymphocytes per 1-ml whole blood simultaneously. Rest of the blood sample was used for biochemical assays. Mononuclear cells were separated from other components of peripheral blood by centrifugation on density gradient media, washed twice and solved in 0.2 ml of PBS. The solution was then labeled with a 10 μl panel of, Peridinin-chlorophyll-protein complex (PerCP), fluorescein isothiocyanate (FITC), and R-phycoerythrin (R-PE) conjugated antibodies anti-CD45 (Becton Dickinson), anti-CD34(Becton Dickinson), and anti- VEGFR2(R&D system), for 20 min at room temperature away from light. After conjugation, red blood cells were lysed by incubating in FACS lysing solution (Becton Dickinson) for 15 min. Cells stained with isotypic controls for IgG1-FITC or R-PE were used as negative controls. After appropriate gating with lymphocytes, $CD45^{low}/CD34^{+}/VEGFR2^{+}$ cells were identified by the dual expression of $CD34^{+}$ and $VEGFR2^{+}$ in the $CD45^{low}$ gates (Fig. 1). The cytometer was set to acquire 100,000 events, and analyses were performed within the lymphocyte gate, in accordance with a technique used by other investigators. Data were processed using the Macintosh CellQuest software program (BD). The absolute count of individual human peripheral blood lymphocytes was performed by

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