Atherosclerosis 269 (2018) 236–244

Contents lists available at ScienceDirect

Atherosclerosis

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

Angiotensin receptor-binding molecule in leukocytes in association with the systemic and leukocyte inflammatory profile

Kotaro Haruhara [a, f], Hiromichi Wakui [a, *], Kengo Azushima [a, b, **], Daisuke Kurotaki [c], Wataru Kawase [c], Kazushi Uneda [a], Sona Haku [a], Ryu Kobayashi [a], Kohji Ohki [a], Sho Kinguchi [a], Masato Ohsawa [a], Shintaro Minegishi [a], Tomoaki Ishigami [a], Miyuki Matsuda [a], Akio Yamashita [d], Hideaki Nakajima [e], Tomohiko Tamura [c], Nobuo Tsuboi [f], Takashi Yokoo [f], Kouichi Tamura [a, ***]

[a] Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[b] Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, Singapore
[c] Department of Immunology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[d] Department of Molecular Biology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[e] Department of Hematology and Clinical Immunology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[f] Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 20 August 2017
Received in revised form
18 December 2017
Accepted 11 January 2018
Available online 12 January 2018

Keywords:
Inflammation
Leukocyte
Non-communicable diseases
Receptor
Renin-angiotensin system

ABSTRACT

Background and aims: The components of the renin-angiotensin system in leukocytes is involved in the pathophysiology of non-communicable diseases (NCDs), including hypertension, atherosclerosis and chronic kidney disease. Angiotensin II type 1 receptor (AT1R)-associated protein (ATRAP) is an AT1R-specific binding protein, and is able to inhibit the pathological activation of AT1R signaling in certain animal models of NCDs. The aim of the present study was to investigate the expression and regulation of ATRAP in leukocytes.

Methods: Human leukocyte *ATRAP* mRNA was measured with droplet digital polymerase chain reaction system, and analyzed in relation to the clinical variables. We also examined the leukocyte cytokines mRNA in bone-marrow ATRAP-deficient and wild-type chimeric mice after injection of low-dose lipopolysaccharide.

Results: The *ATRAP* mRNA was abundantly expressed in leukocytes, predominantly granulocytes and monocytes, of healthy subjects. In 86 outpatients with NCDs, leukocyte *ATRAP* mRNA levels correlated positively with granulocyte and monocyte counts and serum C-reactive protein levels. These positive relationships remained significant even after adjustment. Furthermore, the leukocyte *ATRAP* mRNA was significantly associated with the interleukin-1β, tumor necrosis factor-α and monocyte chemotactic protein-1 mRNA levels in leukocytes of NCDs patients. In addition, the leukocyte interleukin-1β mRNA level was significantly upregulated in bone marrow ATRAP-deficient chimeric mice in comparison to wild-type chimeric mice after injection of lipopolysaccharide.

Abbreviations: ABI, ankle-brachial index; ACE, angiotensin-converting enzyme; ATRAP, angiotensin II type 1 receptor-associated protein; AT1R, angiotensin II type 1 receptor; BM-KO, bone marrow ATRAP-deficient chimeric mice; baPWV, brachial-ankle pulse wave velocity; BM-WT, bone marrow wild-type chimeric mice; CKD, chronic kidney disease; ddPCR, reverse transcription droplet digital polymerase chain reaction; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; IL-1β, interleukin-1β; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; NCDs, Non-communicable diseases; (P)RR, (pro) renin receptor; RAS, renin-angiotensin system; RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; TNF-α, tumor necrosis factor-α; UACR, urinary albumin-to-creatinine ratio; UUO, unilateral ureteral obstruction.

* Corresponding author. Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
** Corresponding author. Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
*** Corresponding author. Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail addresses: hiro1234@yokohama-cu.ac.jp (H. Wakui), azushima@yokohama-cu.ac.jp (K. Azushima), tamukou@med.yokohama-cu.ac.jp (K. Tamura).

https://doi.org/10.1016/j.atherosclerosis.2018.01.013

Conclusions: These results suggest that leukocyte ATRAP is an emerging marker capable of reflecting the systemic and leukocyte inflammatory profile, and plays a role as an anti-inflammatory factor in the pathophysiology of NCDs.

1. Introduction

Non-communicable diseases (NCDs), including hypertension, diabetes, atherosclerosis and chronic kidney disease (CKD), are major health burdens and cause significant mortality and morbidity worldwide. Recent studies have shown that chronic low-grade inflammation via dysregulation of the immune system may play a role in the pathogenesis of NCDs [1–5]. In addition, excessive activation of the renin-angiotensin system (RAS) in local tissue may mediate the development and progression of NCDs, at least in part, by provoking dysregulation of the immune system [6–8].

The existence of functional RAS with its components expression has been proposed in leukocytes and the immune system [9, 10]. In animal studies, angiotensin II type 1 receptor (AT1R) signaling in mouse bone marrow-derived cells and leukocytes affected the inflammatory status and differentiation of immune cells and was implicated in the pathogenesis of angiotensin II-induced hypertension [11,12], subsequent renal injuries [12,13] and unilateral ureteral obstruction (UUO)-induced renal fibrosis [14, 15]. Other previous studies have shown that the activation of angiotensin-converting enzyme (ACE) and renin in bone marrow-derived cells was associated with the development of atherosclerosis [16,17]. Furthermore, exaggerated activation of human leukocyte RAS components, including ACE and (pro) renin receptor ([P]RR), has also been implicated in the pathophysiology of NCDs, as has the RAS in other local tissues and organs [18,19].

We previously identified AT1R-associated protein (ATRAP) as a specific binding protein to AT1R and as a promoting molecule of AT1R internalization [20–26]. In several animal models of NCDs, we showed that the enhancement of local ATRAP expression, such as in the heart, vasculature and kidney, ameliorated tissue injury, probably through the inhibition of hyperactivation of the local tissue AT1R signaling [22–24]. Furthermore, the results of our studies showed that ATRAP deficiency lead to blood pressure elevation in a remnant kidney model and insulin resistance induced by a high-fat diet, concomitant with enhancement of inflammation markers [25, 26]. However, the expression and significance of ATRAP in leukocytes in the physiology and pathophysiology of NCDs have not been evaluated.

Therefore, in the present study, we first examined the ATRAP expression in human leukocytes of healthy subjects. We next analyzed possible relevant clinical factors affecting ATRAP expression in leukocytes of patients with NCDs. Furthermore, we examined the possible effect of ATRAP downregulation on the inflammatory profile of leukocytes in animals after low-dose lipopolysaccharide (LPS) injection, a model of low-grade inflammation in patients with NCDs [27,28], using bone marrow ATRAP-deficient chimeric mice.

2. Materials and methods

2.1. Setting and participants

For the analysis of ATRAP expression in each leukocyte fraction from healthy subjects, volunteers without any diagnosis of a chronic disease were recruited to the present study. For the analysis of clinical factors relevant to ATRAP expression in leukocytes from patients with NCDs, consecutive patients who visited the outpatient clinic at Yokohama City University Hospital, Yokohama, Japan, from April 2015 to March 2016, were recruited. The exclusion criteria were patients who (i) were aged under 19 years, (ii) had a history of corticosteroid or immunosuppressive therapy within the past 3 months, (iii) had been diagnosed with a hematological disease, (iv) were receiving renal replacement therapies and (v) had undergone any adjustments of prescribed medications for hypertension, diabetes or dyslipidemia in the past month.

Healthy subjects and patients with NCDs provided written informed consent for participation. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics review board of Yokohama City University Hospital (No. A150122002) and was entered into the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR, www.umin.ac.jp/ctr/index/htm/, UMIN000016846).

2.2. Separation of leukocyte fractions

Approximately 20 mL of whole blood was collected in heparin-coated tubes and incubated with hemolysis solution (BD Pharm-Lyse; BD Biosciences, San Jose, CA) for 10 min at room temperature. After being washed, cells were incubated with 10% human AB serum (Access Biologicals, Vista, CA) for 20 min at 4 °C [29, 30]. For surface marker staining, cells were incubated for 30 min at 4 °C with appropriately diluted antibodies. Granulocytes were separated with FITC-*anti*-CD66b and PE-*anti*-CD16 (Biolegend, San Diego, CA). Monocytes and B- and T-lymphocytes were fractionated with FITC-*anti*-CD19, PE-*anti*-CD14, and APC/Cy7-antiCD3 (Biolegend). Granulocytes, monocytes, B-lymphocytes and T-lymphocytes were defined as CD66b+/CD16+, CD14+/CD3-/CD19-, CD14-/CD3-/CD19+ and CD14-/CD3+/CD19- cells, respectively. 7-aminoactinomycin D (Sigma-Aldrich, St. Louis, MO) was used to exclude dead cells. Cell fractions were separated on a BD FACSAria II (BD Biosciences). Total RNA was extracted using the RNeasy Micro Kit (QIAGEN, Germantown, MD), and cDNA was synthesized from 28.5 ng of total RNA using the PrimeScript RT reagent Kit (Takara-Bio, Shiga, Japan).

2.3. Gene expression analyses by reverse transcription droplet digital polymerase chain reaction and real-time quantitative reverse transcription PCR

Approximately 2.5 mL of whole blood was collected in PAX gene Blood RNA Tubes (Nippon Becton Dickinson Company, Tokyo, Japan). Total leukocyte RNA was extracted using the PAX gene Blood RNA Kit (QIAGEN) for reverse transcription, and leukocyte cDNA was synthesized from 0.25 μg of total RNA using the SuperScript III First-Strand System (Invitrogen, Carlsbad, CA).

Recent developments in the quantitative analysis of gene expression by reverse transcription droplet digital polymerase chain reaction (ddPCR) might provide an opportunity to reduce the quantitative variability seen using real-time quantitative reverse transcription PCR (RT-qPCR), particularly in human samples of