Receptor for advanced glycation end products (RAGE), inflammatory ligand EN-RAGE and soluble RAGE (sRAGE) in subjects with Takayasu's arteritis

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Takayasu's arteritis (TA) is a rare primary and granulomatous large vessel vasculitis of unknown origin that predominantly affects the aorta and its major branches. It has been shown to affect individuals of any age, gender and a wide variety of ethnic and racial population worldwide. The pathophysiology of TA is complex and multi-factorial and the exact pathogenesis remains to be elucidated; however, participation of autoimmunity, inflammation and oxidative stress has potentially been implicated [1–3].

Receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily and is expressed on the surface of various cells e.g. endothelium, mononuclear phagocytes, lymphocytes and smooth muscle cells. Extracellular newly identified RAGE-binding protein (EN-RAGE or S100A12), an inflammatory ligand of RAGE, is a member of the S100 protein family. Since its discovery two decades back [4], the inflammatory role of RAGE and EN-RAGE interaction is now well established in various chronic inflammatory disorders. RAGE has a circulating truncated variant isoform and soluble RAGE (sRAGE), corresponds to its extracellular domain only. By competing with cell-surface RAGE for ligand binding, sRAGE contributes to the removal/neutralization of circulating ligands, thus, functioning as a decoy [5–7]. Previously, we demonstrated low sRAGE levels showing inverse correlation with MMPs in a group of forty TA subjects as compared to controls [8].

Keeping in mind the importance of RAGE biology in various diseases, we thought it worth to determine the expression of RAGE and its inflammatory ligand EN-RAGE at transcriptional level in peripheral blood mononuclear cells (PBMCs) from TA subjects. For this, 24 subjects with TA and 24 normal healthy controls were recruited as described previously [3,8,9]. The Institutional Ethics Committee approved the present study. Venous blood was collected from overnight fasted individuals in the morning from antecubital vein and processed as described previously [5,8]. Semi-quantitative RT-PCR was performed for determining the transcriptional expression of RAGE and EN-RAGE in PBMCs [5]. Circulating levels of sRAGE were determined in all the study subjects using commercially available enzyme-linked immunoassay as per manufacturer's instructions (DRG00, R&D Systems, USA). Statistical analysis was performed using Prism Statistical Software.

Anthropometric features of the subjects are shown in Table 1. We observed no significant difference between lipid and lipoprotein levels in TA subjects and their controls ($p>0.05$, data not shown). The transcriptional expression of RAGE and EN-RAGE genes was found to be significantly higher in blood mononuclear cells isolated from TA subjects with active disease as compared to their control counterparts (Fig. 1A–C). However, expression of both the genes did not show any significant difference in subjects with active disease versus those who were in remission ($p>0.05$), though the levels were higher in TA subjects with active disease. However, serum sRAGE levels were observed to be significantly lower in patients with active TA when compared to the controls ($p<0.001$) as well as compared to TA subjects in remission ($p<0.001$) (Fig. 1D). Further, a positive and significant correlation between RAGE and EN-RAGE mRNA expression was also observed in subjects with TA ($r=0.531$; $p=0.003$, Fig. 1E). However, no significant correlation of sRAGE levels was observed with either RAGE or EN-RAGE mRNA in TA subjects.

This is the first report in literature which demonstrates that mRNA levels of RAGE and its ligand EN-RAGE are up-regulated in subjects with TA. This study also demonstrates a positive correlation of RAGE and EN-RAGE, in subjects with TA for the first time. Our data is supported by other reports, which demonstrate that RAGE expression increases stably in certain pathological processes, and is associated with ligand-rich microenvironments [6,7]. In a previous study, we demonstrated that RAGE and EN-RAGE genes are augmented in non-diabetic CAD subjects and showed their positive correlation with the severity of disease [5]. Ligation of this receptor with its ligands activates the downstream signaling pathways such as NF-κB [10]. In addition, RAGE and EN-RAGE interactions could further induce the expression of adhesion molecules, migration and proliferation of leukocytes and augment cytokine release from the lymphocytes. Excessive expression of these genes at sites of inflammation may feasibly form a positive feedback loop that may further aggravate underlying inflammation. In corroboration to this, other reports in literature and our work have clearly demonstrated a potential participation of various cytokines, chemokines and MMPs in the pathophysiology of TA [3,8,9,11]. Significantly increased RAGE–EN-RAGE expression in TA further confirms a prevalent oxidative stress and inflammatory milieu in such a situation.

Presence of various functional splice variants of RAGE is another important characteristic of this particular receptor. The production of endogenous secretory RAGE by alternative splicing may influence the actions of ligands on the full form of RAGE, located on the plasma membrane [12]. Finding increased RAGE mRNA levels in TA subjects, one

Table 1
Baseline characteristics of the study subjects.

Parameter	Controls (n = 24)	TA (n = 24)	TA	
			Active disease (n = 17)	In remission (n = 7)
Age (years)	32 ± 11	32 ± 11	32 ± 10	31 ± 10
Sex ratio (M:F)	6:18	4:20	3:14	1:6
SBP (mm Hg)	118 ± 7	134 ± 22	138 ± 23	121 ± 13
DBP (mm Hg)	77 ± 7	83 ± 15	85 ± 17	77 ± 7
BMI (kg/m^2)	21.85 ± 3.32	21.23 ± 2.65	21.14 ± 2.49	21.44 ± 3.22

n = number of subjects; SBP—systolic blood pressure; DBP—diastolic blood pressure; Results are expressed as mean ± S.D.

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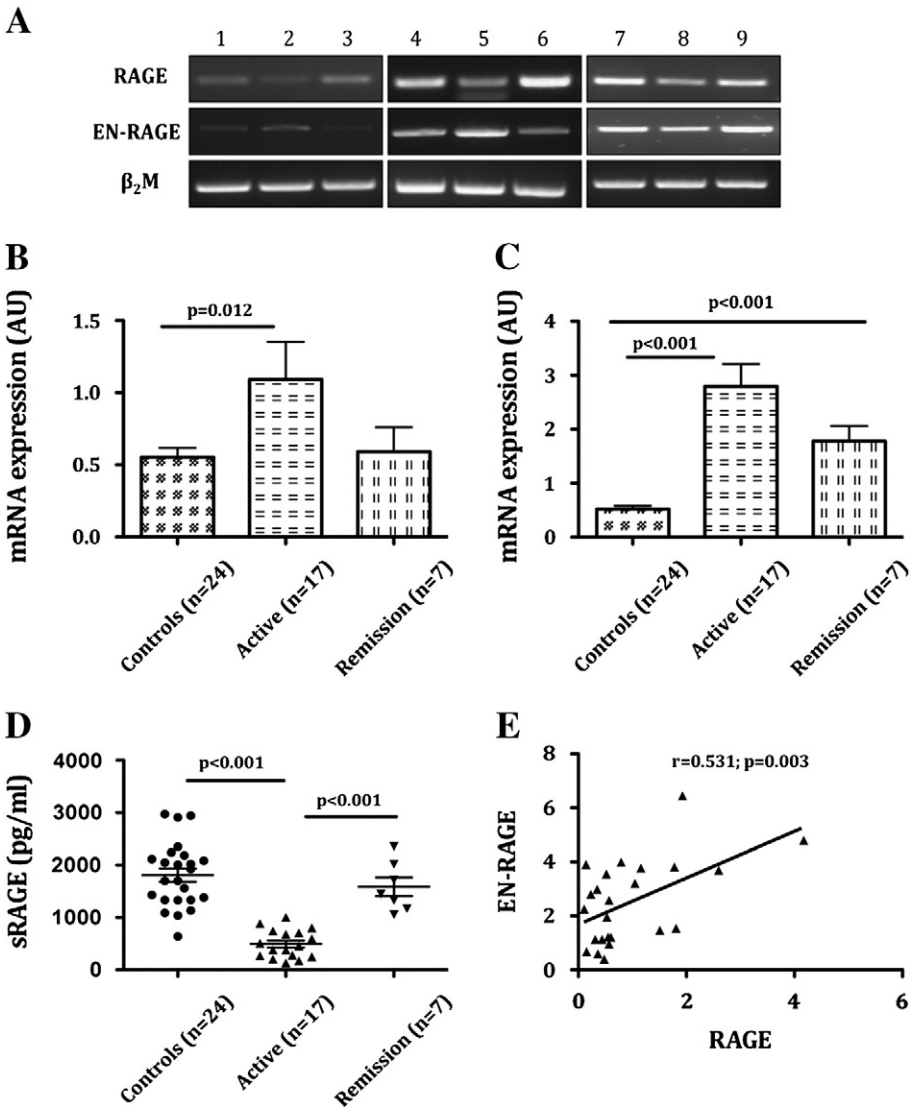

Fig. 1. A. Representative gel pictures of PCR amplified products for RAGE, EN-RAGE and β_2M. Lanes 1–3: Healthy controls, lanes 4–6: TA subjects with active disease, lanes 7–9: TA subjects in remission. B–C. Depicts the mean ± SEM mRNA expression of RAGE (B) and EN-RAGE (C) in PBMCs of study subjects as determined by semi-quantitative RT-PCR and depicted as arbitrary units (AU). D. Vertical scatter plot of sRAGE levels in study individuals as measured by immunoassay. Horizontal line depicts the mean ± SEM in each group. E. Depicts a positive correlation between EN-RAGE and RAGE mRNA expression in TA subjects.

would also expect an increase in sRAGE levels as well, however we did not observe the same. A possible explanation could be that the relative abundance of RAGE isoforms may vary accordingly in different cells and tissues and among individuals and/or conditions [12]. Another possible reason could be that full-length form of RAGE mRNA levels might have majorly increased and not the others. However, role of epigenetic or post-translational modifications of full length RAGE i.e. shedding of ectodomain and proteolysis by MMP-9 or ADMA10, cannot be ignored [13]. It is also possible that at the same time shedding may be modulated by binding of pro-inflammatory ligands (e.g. EN-RAGE) or alternatively, sRAGE interacts with ligands and in the form of complex.

Observations of the present study as well as previous studies highlight the fact that RAGE and EN-RAGE axis might have joined together with pro-inflammatory cytokines and therefore, contribute to disease pathognomonics [6,7]. To keep an eye on or inhibiting RAGE–EN-RAGE interaction in early stages of the inflammatory process may inhibit inflammation and therefore, can prevent further sequels. Caution must be taken before extrapolating these results as we have studied PBMCs as cellular models for gene expression studies and RAGE is also expressed by a variety of other cells such as endothelial cells and smooth muscle cells, the cell types that are relevant to the pathophysiology of TA.

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Door to balloon time: How short is enough under highly accessible nationwide insurance coverage? Analysis from the Japanese multicenter registry

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Over the past decades, primary percutaneous coronary intervention (PCI) has emerged as an effective treatment strategy for acute ST-segment-elevation myocardial infarction (STEMI), and time to treatment has a great impact on survival. Clinical practice guidelines recommend that door-to-balloon (DTB) should not exceed 90 min [2] and American College of Cardiology(ACC)launched the DTB Alliance, a national campaign to improve DTB by advocating the adoption of key strategies that have been shown to reduce delays [3]. As a consequence, the DTB value declined from a median of 96 min in the year 2005, to a median of 64 min in the year 2010 [4].

The impact of DTB is not clear in Japan, where primary PCI facilities are readily available in a relatively small geographical area, enabling more timely access. In addition, Japan achieved universal health insurance coverage in 1961. Virtually the entire population is covered by health plans through social health insurance [5]. Consequently, the network of ambulances is thoroughly developed in Japan. These differences may change the effect of DTB. Recently, Kimura et al. evaluated the impact of onset-to-balloon and DTB, and found that the benefit of short DTB was limited to patients with early presentation [6].

The purpose of this study was to assess the impact of delays in DTB and time to presentation on detailed in-hospital complications (e.g.., bleeding or contrast-induced nephropathy [CIN]) in STEMI patients in Japan.

We analyzed data from the Japan Cardiovascular Database (JCD)-KICS with patients registration between September 2008 and 2011. The JCD is an ongoing, prospective, multicenter cohort study designed to collect clinical background and outcome data on PCI among Japanese patients. The participating hospitals record patient data into an internet-based database. Dedicated clinical coordinators check the system constantly to ensure that the reported data are complete and internally consistent.

Among 3898 patients enrolled in the JCD-KICS registry, 834 patients had an admission diagnosis of STEMI and underwent PCI within 24 h of onset, excluding 253 patients with cardiogenic shock or cardiopulmonary arrest. We excluded 620 patients for whom the time to presentation or DTB was not known. We investigated the remaining 214 patients. DTB was defined as the time from hospital arrival until the first balloon inflation. Time to presentation was defined as the time from symptom onset until arrival at the hospital.

The collected outcomes were in-hospital mortality, shock as a complication, bleeding complication, CIN, and TIMI flow of <3. The principal independent variables were DTB and time to presentation. According to DTB, patients were divided into 2 groups (DTB of <90 min and of ≧90 min). According to time to presentation, patients were divided into 2 groups (time to presentation of <2 h and 2–24 h).

Median time to presentation was 196 min (interquartile range 0 to 255 min) and median DTB was 104 min (interquartile range 60 to 120.5 min). A comparison of baseline variables in long versus short DTB, and late versus early presentation is shown in Table 1 and Table 2, respectively. There were no significant differences between these groups.

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