Inverse Association between Cardiac Troponin-I and Soluble Receptor for Advanced Glycation End Products in Patients with Non-ST-Segment Elevation Myocardial Infarction

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ABSTRACT

Interaction of advanced glycation end products (AGEs) with the receptor for advanced AGEs (RAGE) results in activation of nuclear factor kappa-B, release of cytokines, expression of adhesion molecules, and induction of oxidative stress. Oxygen radicals are involved in plaque rupture contributing to thromboembolism, resulting in acute coronary syndrome (ACS). Thromboembolism and the direct effect of oxygen radicals on myocardial cells cause cardiac damage that results in the release of cardiac troponin-I (cTnI) and other biochemical markers. The soluble RAGE (sRAGE) compete with RAGE for binding with AGE, thus functioning as a decoy and exerting a cytoprotective effect. Low levels of serum sRAGE would allow unopposed serum AGE availability for binding with RAGE, resulting in the generation of oxygen radicals and proinflammatory molecules that have deleterious consequences and promote myocardial damage. sRAGE may stabilize atherosclerotic plaques. It is hypothesized that low levels of sRAGE are associated with high levels of serum cTnI in patients with ACS. The main objective of the study was to determine whether low levels of serum sRAGE are associated with high levels of serum cTnI in ACS patients. The serum levels of sRAGE and cTnI were measured in 36 patients with non-ST-segment elevation myocardial infarction (NSTEMI) and 30 control subjects. Serum levels of sRAGE were lower in NSTEMI patients (802.56 ± 39.32 pg/mL) as compared with control subjects (1311.43 ± 66.92 pg/mL). The levels of cTnI were higher in NSTEMI patients (2.18 ± 0.33 µg/mL) as compared with control subjects (0.012 ± 0.001 µg/mL). Serum sRAGE levels were negatively correlated with the levels of cTnI. In conclusion, the data suggest that low levels of serum sRAGE are associated with high serum levels of cTnI and that there is a negative correlation between sRAGE and cTnI.

KEYWORDS: Soluble receptor for advanced glycation end products, cardiac troponin-I, non-ST-elevation myocardial infarction, cytokines, oxidative stress, thromboembolism

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The biomarker cardiac troponin I (cTnI) is a protein that leaks out of injured/infarcted myocardial cells through their damaged cell membranes into the bloodstream within 4 to 6 hours of a myocardial infarction and remains elevated for up to 2 weeks.^{1,2} Increased serum cTnI levels in patients with acute coronary syndrome (ACS) differentiate and guide the management of patients with unstable angina (normal cTnI levels) and non-ST-segment elevation myocardial infarction (NSTEMI; increased cTnI levels).³⁻⁷ cTnI is elevated in both NSTEMI and ST-segment elevation myocardial infarction (STEMI) patients.⁸ NSTEMI is suspected when the levels of cTnI are greater than 0.4 µg/L.^{8,9}

Advanced glycation end products (AGEs) are a heterogeneous group of irreversible adducts resulting from the nonenzymatic glycation and oxidation of proteins, nucleic acids, and lipids.^{10,11} Receptors for AGEs (RAGE) have three isoforms that include full-length RAGE, N-truncated RAGE, and C-truncated soluble RAGE (sRAGE).¹¹⁻¹⁴ The interaction of full-length RAGE with AGEs increases the expression of the cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-6, and IL-8],^{11,15,16} activates nuclear factor kappa B,¹⁵ which in turn increases the expression of proinflammatory injury genes for cytokines¹¹ and increases the generation of oxygen radicals.^{17,18} IL-6 and TNF-α are known to activate granulocytes to generate oxygen radicals.¹⁹⁻²¹ TNF-α also stimulates NADPH-oxidase in the endothelial cells to generate oxygen radicals.^{22,23} Oxygen radicals act upon unsaturated fatty acids of the phospholipid cell membrane to produce the lipid peroxide malondialdehyde, which increases membrane fluidity and permeability with a loss of membrane integrity.^{24,25} Oxygen radicals have been implicated in ischemia-reperfusion cardiac injury.²⁶⁻²⁹ Ischemia-reperfusion injury is associated with increases in the serum biomarker creatine kinase and the myoglobin fraction of creatine kinase.³⁰ Oxygen radicals are involved in the release of the biomarker lactic acid dehydrogenase from cultured endothelial cells³¹ and lysosomal enzymes from isolated lysosomes.³²

sRAGE circulates in plasma¹³ and acts as a decoy for RAGE ligands by competing with full-length RAGE for ligand binding.³³ It plays a cytoprotective role by preventing the interaction of RAGE with AGEs. Low levels of serum sRAGE would promote uninhibited increased interaction of AGEs with RAGE, leading to an increased production of oxygen radicals that would cause cellular damage and the release of cTnI into the circulation.

Dysregulation of extracellular matrix metabolism primarily from local overexpression of matrix metalloproteinases (MMPs)³⁴ may weaken atherosclerotic plaques, causing their rupture.³⁵⁻³⁷ Plaque rupture and thromboembolisms cause the majority (~90%) of myocardial infarctions.³⁸ Atherosclerotic plaque contains

lipid-laden foam cells that release oxygen radicals leading to activation of MMPs, which augment plaque instability.^{35,39} In humans, RAGE overexpression is associated with an enhanced inflammatory reaction and cyclooxygenase-2 and prostaglandin E synthetase-1 expression in plaque macrophages. This effect may augment plaque destabilization by promoting MMP expression.⁴⁰ Oxygen radicals have been shown to produce endothelial injury.³¹ Two major causes of coronary thrombosis and hence ACS are plaque rupture and endothelial erosion. It has been reported that simvastatin suppresses RAGE expression by decreasing myeloperoxidase-dependent AGE generation, resulting in plaque stabilization by inhibiting biosynthesis of prostaglandin-2-dependent MMPs.⁴¹

It is hypothesized that low levels of serum sRAGE will be associated with high serum levels of cTnI in patients with ACS. Therefore, the serum levels of sRAGE and cTnI were measured in patients with NSTEMI.

METHODS

Study Subjects

The study comprised 36 consecutive male NSTEMI patients who were admitted to the Royal University Hospital, Saskatoon, Saskatchewan. These patients had no history of diabetes, inflammatory diseases, or smoking. The control group consisted of 20 age-matched subjects with no history of angina, heart disease, diabetes, inflammatory disease, or history of smoking. Blood samples were collected from patients and control subjects for the measurement of cTnI and sRAGE. The Ethics Committee for Human Studies at the University of Saskatchewan and the Saskatoon Health Region approved the protocol.

Written informed consent was obtained from each patient and control subject.

Biochemical Measurements

Serum levels of sRAGE and cTnI were measured in blood samples collected from patients and control subjects. sRAGE was measured using commercially available enzyme linked immunoassay kits (R&D Systems, Minneapolis, MN). cTnI was measured on an automated Abbott AxSYM (Abbott Laboratory, Abbott-Park, IL).

RESULTS

sRAGE in NSTEMI Patients and Control Subjects

The serum levels of sRAGE in 36 NSTEMI patients and 20 control subjects are summarized in Fig. 1. The

Figure 1 Serum levels of soluble receptor for advanced glycation end products (sRAGE) in control subjects and in patients with non-ST-elevation myocardial infarction (NSTEMI). Results are expressed as mean $\pm$ standard error. * $p < 0.05$, control versus NSTEMI.

serum levels of sRAGE were lower in NSTEMI patients as compared with control subjects (802.56 ± 39.32 versus 1311.43 ± 66.92 pg/mL).

cTnI in NSTEMI Patients and Control Subjects

The serum cTnI levels in 36 NSTEMI patients and 20 control subjects are summarized in Fig. 2. The serum levels of cTnI were higher in NSTEMI patients compared with control subjects (2.18 ± 0.33 versus 0.012 ± 0.001 μ g/mL).

Figure 2 Serum levels of cardiac troponin-I (cTnI) in control subjects and in patients with non-ST-segment elevation myocardial infarction (NSTEMI). Results are expressed as mean $\pm$ standard error. * $p < 0.05$, control versus NSTEMI.

Figure 3 Correlation of soluble receptor for advanced glycation end products (sRAGE) with cardiac troponin-I (cTnI) in patients with non-ST-segment elevation myocardial infarction.

Correlation between sRAGE and cTnI

A correlation between serum sRAGE and cTnI in patients with NSTEMI is shown in Fig. 3. The levels of serum sRAGE were negatively correlated with cTnI with a p value of 0.038. The results suggest that decreases in serum sRAGE are associated with increases in cTnI.

DISCUSSION

This is the first study to show that high levels of serum cTnI are associated with low levels of serum sRAGE in NSTEMI patients. Low levels of serum sRAGE have been reported in patients with coronary disease.^{33,42} Elevated serum levels of cTnI have also been reported in patients with ACS (STEMI and NSTEMI).³⁻⁹ The present findings of elevated cTnI in NSTEMI patients are consistent with the findings of other investigators. High serum levels of cTnI in NSTEMI could be due to myocardial damage induced by coronary thrombosis. Major causes of coronary thrombosis are plaque rupture and endothelial erosion. RAGE-AGE axis may be involved in clot formation, plaque rupture, and endothelial erosion. Low levels of sRAGE in these patients would increase levels of AGEs and its unopposed interaction with RAGE, resulting in increased production of cytokines^{11,15,16} and reactive oxygen species.^{17,18} Reactive oxygen species activate MMPs,³⁹ the overexpression of which weakens the atherosclerotic plaque and produces plaque rupture.^{35,36,39,43} Cytokines have been shown to be involved in plaque rupture.^{44,45} Increases in serum cTnI in NSTEMI patients may also be due to enhanced oxygen radical production, which could damage the myocardium, thus releasing cTnI into the circulation. Increased levels of oxygen radicals have been reported in patients with ACS.^{46,47} The cellular membrane damage from oxygen radicals^{24,25,31} may result in the release of various biomarkers including cTnI.

A negative correlation between sRAGE and cTnI suggests that the severity of cardiac damage varies inversely with the levels of sRAGE.

In conclusion, the data suggest that the serum levels of sRAGE are inversely related to the serum levels of cTnI in NSTEMI patients and that the levels of sRAGE may determine the severity of myocardial infarction.

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