Advanced Glycation End Products:Receptors for Advanced Glycation End Products Axis in Coronary Stent Restenosis: A Prospective Study

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Abstract

Keywords

- PCI
- stent
- restenosis
- cardiac Biomarkers
- advanced glycation end products
- soluble receptor for advanced glycation end products
- oxidative stress

This study was aimed to correlate the pre- and 6-month postpercutaneous coronary intervention (PCI) serum concentrations of advanced glycation end products (AGE), soluble receptors for advanced glycation end products (sRAGE), AGE/sRAGE ratio, and serum malondialdehyde (MDA) levels with in-stent restenosis (ISR) among patients receiving either a drug-eluting stent (DES) or a bare-metal stent (BMS). In-stent restenosis remains as an adverse outcome following PCI. Sixty consecutive nondiabetic, Caucasian male patients, diagnosed with a non-ST-elevation myocardial infarction who received either a DES or BMS via PCI, were enrolled. Baseline levels of serum AGE, sRAGE, AGE/sRAGE ratios, MDA, and angiographic parameters were determined at stenting and at 6 months. Patients with and without ISR at 6 months were compared on both baseline and 6-month biomarker levels and within stent types. The pre-PCI serum AGE levels and AGE/sRAGE ratios were higher in ISR patients compared with non-ISR patients, while the pre-PCI and post-PCI serum sRAGE levels were lower in ISR patients compared with non-ISR patients. The pre and post-PCI levels of MDA were also higher in ISR patients. Comparing stent types, relative levels of MDA between those with and without ISR at the respective time points were similar, although changes between time points appeared type specific. Post-PCI ISR correlates with low serum values of sRAGE and high serum values of AGE, MDA, and AGE/sRAGE ratio which are present at stenting. The associations of baseline AGE, sRAGE, AGE/sRAGE, and MDA levels with ISR appear consistent between stent types.

In-stent restenosis (ISR) remains a major complication following percutaneous coronary intervention (PCI)¹ and is defined as a reoccurring diameter stenosis > 50% at the stent segment or its edge.² PCI utilizing drug-eluting stents (DES) or bare-metal stents (BMS) have restenosis incidences of approximately 5.5 and 24.4%, respectively.² McNair et al reported restenosis in 41% of nondiabetic, non-ST-elevation myocardial infarction BMS recipients.³ Although attenuated with DES, ISR persists in both stent types.

Glycation is the nonenzymatic incubation of sugars with proteins, lipids, and/or nucleic acids forming early glycation products. Through a series of rearrangements and oxidative reactions second stage advanced glycation end products (AGE) arise.⁴ The receptor for advanced glycation end products (RAGE) is cell bound and its activation has been associated with cardiovascular disease.^{5–7}

Reactive oxygen species (ROS) generation under pathological conditions may promote the harmful effects of

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peroxidation of lipid membranes that may cause damage in biological systems and induce atherogenesis.⁵ Specifically, the AGE:RAGE interaction in endothelial cells activates the production of ROS which are involved in signal transduction pathways to induce the expression of response to injury genes that are involved in the production of proinflammatory cytokines and adhesion molecules which participate in the pathogenesis of the atherosclerotic lesion.⁶ This interaction has been implicated in balloon injury and de-endothelialization-induced neointimal hyperplasia in animal models,^{7,8} while soluble RAGE (sRAGE) administration decreases this response.^{7,8} Our laboratory has previously shown that in patients receiving BMS both low serum sRAGE levels and high AGE/sRAGE ratio have predictive value for post-PCI restenosis.³ Elevated serum AGE levels have been shown to independently predict ISR 6 months post-DES implantation in diabetic patients, potentially foretelling adverse outcome.⁹ As AGE, RAGE, and sRAGE relate to vascular injury, their levels may predict vascular complications post-PCI. RAGE, a cell surface protein on the endothelium, cannot be measured. However, sRAGE and AGE serum levels are quantifiable. Since the interaction of RAGE with AGE results in increased generation of ROS and expression of cytokines and adhesion molecules,^{4,10,11} substitutionary binding of sRAGE to AGE may allay these processes. Thus, low sRAGE and high AGE/sRAGE ratio levels potentially associate with increased ROS levels. The adverse effects of oxidative stress include cell membrane damage and lipid membrane peroxidation, precipitating reduced vasomotor activity.¹² Malondialdehyde (MDA) is an advanced oxidation product and a recognized oxidative stress biomarker.¹³ Therefore, the objective of measuring MDA is that it serves as an indirect biomarker of ROS activity.¹⁴

Collectively, endothelial injury, sRAGE, AGE, and ROS appear predictive of restenosis. In this pilot study, we hypothesize that post-PCI ISR is associated with low serum levels of sRAGE, high serum levels of AGE, and high AGE/sRAGE ratios. A positive correlation is expected between levels of AGE and AGE/sRAGE with oxidative stress (MDA) and a negative correlation between levels of sRAGE and MDA. We also wish to explore if these associations differ by stent type.

Materials and Methods

Patient Sample

Sixty nondiabetic, Caucasian, male patients diagnosed with acute coronary syndrome, non-ST-elevation myocardial infarction (NSTEMI) by the attending cardiologist who provided written consent. Recruitment occurred between November 2, 2015 and January 11, 2016. The University of Saskatchewan Biomedical Research Ethics Board approved the study protocol and the Provincial Health Authority granted operational approval. Patients were separated into two groups based upon the stent type selected by the cardiologist: Group I ($n = 30$) received a drug-eluting stent (DES) and Group II ($n = 30$) received a bare-metal stent (BMS). Admitted patients scheduled for a planned PCI were consecutively recruited until group quotas of 30 subjects were met.

The inclusion criteria were as follows: (1) admitted, planned PCI, acute coronary syndrome patients with NSTEMI; (2) discrete de novo localized lesions (single or double vessels); (3) reference artery diameter ≥ 2.5 mm; (4) BMS or DES implantation; (5) age 50–70 years; and (6) male sex. The exclusion criteria were as follows: (1) acute myocardial infarction of more than 5 days prior; (2) post-bypass graft surgery; (3) patients with more than two stent implantations; (4) history of inflammatory disease; (5) history of valve disease; (6) Alzheimer's disease; and (7) current smokers.

A sample size of 30 subjects per stent type was set prior to study commencement. Assuming restenosis rates of 5 and 40% for DES and BMS, respectively,² 13 restenosis cases were expected ($30 \times 0.40 + 30 \times 0.05 = 13.5$). Utilizing published mean and standard deviation (SD) values of pre-PCI AGE, sRAGE, and AGE/sRAGE from a similar population,³ and assuming two-sided testing with $\alpha = 0.05$ and power = 0.80, the largest number of ISR outcomes required per group was 12. As such 30 subjects per stent type were deemed adequate. SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was used for these calculations.

Data Collection and Patient Classification

Demographic data and clinical characteristics were collected at baseline. In the cardiac catheterization laboratory, a 5 or 6-French introducer was inserted percutaneously into the right femoral or radial artery and blood was drawn for analysis prior to angiography. All subjects returned 6 months post-PCI for repeat angiography with blood again drawn immediately after arterial access. The groups were then further subdivided based upon the accepted definition of restenosis. Binary angiographic restenosis was defined as $\geq 50\%$ stenosis at follow-up or the loss of $>50\%$ of the initial gain located between the proximal and distal reference segments of the affected vessel (i.e., ISR).¹ The classifications were ISR or non-ISR, cross-classified as DES ISR, DES non-ISR, BMS ISR, or BMS non-ISR.

Medical Management

Standard antiplatelet therapy prescribed prior to PCI administered: (1) aspirin, with a loading dose of 162 mg, and then 81 mg/day for life and (2) clopidogrel, 300 or 600 mg then 75 mg/day for up to 1 year. Additional medical therapy was at the discretion of the attending cardiologist.

Stent Types

All DES released everolimus (XienceXpedition V and Xience Prime LL, Abbott Vascular Division, Lake-Bluff, IL or PROMUS Premier stent Boston Scientific, 300 Boston Scientific Way Marlborough, MA). The Medtronic Integrity (Medtronic, Inc., Santa Rosa, CA, Medtronic.com) or the BIOTRONIK PRO-Kinetic Energy (BIOTRONIK AG, Bulach, Switzerland) were the BMS used.

Angiographic Analysis

Coronary angiograms were analyzed using a semiautomated edge contour detection computer analysis system (Q CACMS version 4, Medis Medical Imaging Systems, Inc., Leiden, the Netherlands) by two interventional cardiologists blinded to

the patients' clinical characteristics. The reference diameter, mean lumen diameter, percentage diameter stenosis, and lesion length were determined pre-PCI, immediately post-PCI, and 6-month post-PCI. Acute gain was defined as the difference between the mean lumen diameter pre-PCI and immediately post-PCI. Late loss occurred between the intervention and 6-month follow-up angiogram calculated as the difference between mean lumen diameter at 6-month follow-up and immediately post-PCI. Net gain is the difference between acute gain and late luminal loss.¹⁵ In patients who had received more than one stent at baseline, only the culprit lesion was evaluated.

Biochemical Measurements

Blood was collected for cholesterol, high-sensitivity troponin T, creatinine, MDA, AGE, and sRAGE measurement. Serum total sRAGE levels were measured using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol. Serum AGE was also measured using the competitive ELISA method (Cell Biolabs, Inc., San Diego, CA). MDA is an end product of lipid peroxidation that reacts with thiobarbituric acid (TBA) to form a colored substance. Measurement of MDA by TBA reactivity is the most widely used method for assessing lipid peroxidation. Thiobarbituric acid reactive substance (TBARS) were estimated by the method of Yagi.¹⁶ The Cobas C501 chemistry analyzer (Roche Hitachi, Holliston, MA) was used to measure the cholesterol panel. While a Cobas E601 (Roche Hitachi, Holliston, MA) chemistry analyzer was used to evaluate high-sensitivity troponin T levels. AGE, sRAGE, AGE/sRAGE ratio, and MDA were reassessed at 6-month post-PCI.

Statistical Analysis

The study subjects were described, both overall and stratified by 6-month ISR status. As several variables of interest were found to violate normality and the number of subjects in the ISR group was relatively small, a nonparametric approach was applied (medians, interquartile ranges [IQR]). Baseline demographics, physical/echocardiographic findings, biochemical levels, and angiographic measurements were compared between the ISR and non-ISR groups with key variables reassessed at 6 months. For these evaluations, continuous and categorical variables were respectively compared between the groups using the Wilcoxon's rank sum test and Chi-square test. Additionally, within each group, the within-subject changes in AGE, sRAGE, AGE/sRAGE ratio, and MDA between baseline and 6 months were evaluated using the Wilcoxon's signed-rank test.

Additional analysis evaluated biomarker changes using the above methods under additional stratification by stent type, this allowed further comparison within specific stent types at baseline and 6 months. The correlation between MDA levels and AGE, sRAGE, and AGE/sRAGE ratio levels respectively were assessed using Spearman's correlation. Lacking randomization, BMS, and DES recipients were compared to ensure that observed marker differences in between stent types were not potentially attributable to clinical differences. The *t*-test was used for these comparisons.

All statistical testing was two-sided and α was set at 0.05, although Bonferroni correction was applied within each endpoint to account for multiple comparisons made between groups (baseline and 6-month testing; corrected $\alpha = 0.05/2 = 0.025$) and also when testing within two separate groups (corrected $\alpha = 0.05/2 = 0.025$). All computations utilized the software R (R Core Team [2017], R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographic and Clinical Characteristics

Subject characteristics are shown in ►Table 1. Examining the 12 individuals who developed ISR and the 48 who did not, there were no statistically significant risk factor differences. Creatinine clearance was slightly higher in the ISR group (median values 88.5 vs. 74.0 mL/min/1.73 m²; $p = 0.02$). As expected, more BMS subjects developed ISR ($n = 8$, 27% vs. $n = 4$, 13%) although this difference was not statistically significant. No other notable differences were found.

Quantitative Coronary Angiography Results

The pre-PCI, post-PCI, and 6-month follow-up values for quantitative coronary angiography in-stent lesion characteristics are summarized in ►Table 2. ISR and non-ISR subjects had comparable diseased vessels pre-PCI, which were similar in size (median reference diameter 3.0 vs. 2.9 mm) and narrowing (median percent stenosis 74.5 vs. 73.5%). Only regarding involved segment length were subjects with subsequent ISR slightly different at 19.5 (IQR 18.8, 19.8) versus 18.4 mm (IQR 17.6, 19; $p = 0.02$).

As all patients underwent the same procedure, immediate post-PCI measurements were consistent; median values for mean lumen diameter in non-ISR and ISR patients were 2.7 and 2.6 mm, respectively. Stenosis was similarly reduced, with median acute gains in mean lumen diameter of 0.51 and 0.49 mm. However, over 6 months, the median loss in ISR subjects of 1.06 mm was substantially more than the median 0.23 mm loss in non-ISR subjects. Similarly, the mean lumen diameter was much narrower in ISR subjects (median values of 1.4 vs. 2.6 mm). Non-ISR subjects had a median overall increase (i.e., net gain) of 0.25 mm from initial mean pre-procedure diameter, while restenosis subjects typically worsened beyond their initial narrowing (median net gain of −0.58 mm).

Biochemical Measurements

Median group AGE, sRAGE, AGE/sRAGE ratio, and MDA levels, along with IQR's and *p*-values for group comparisons are presented in ►Table 3. Median within-subject changes in these biomarkers between the time points are available in supporting information, ►Table S1 (available only in the online version), although associated *p*-values are included in ►Figs. 1 and 2.

AGE, sRAGE, and AGE/sRAGE Ratio Levels

At baseline, median serum AGE levels were significantly higher in ISR subjects versus non-ISR subjects (►Fig. 1A); at 6 months,

Table 1 Demographic and biochemical characteristics of ISR and non-ISR patients

Variables	All subjects	Non-ISR	ISR	p-Value ^a
	(n = 60)	(n = 48)	(n = 12)	
Age, y; median (IQR)	57 (52.8, 60)	56.5 (53, 60)	58 (51.5, 63.3)	0.58
BMI, kg/m ² , median (IQR)	28.5 (25.9, 32.1)	28.9 (25.9, 32.3)	27.9 (25.9, 29.1)	0.25
Ejection fraction, %, median (IQR)	55 (47.8, 64)	55 (45, 64)	60 (53.8, 63.3)	0.16
Stent type, n (%),				
Bare-metal stent	30 (50.0)	22 (73.3)	8 (26.7)	0.33
Drug-eluting stent	30 (50.0)	26 (86.7)	4 (13.3)	
Creatinine clearance, mL/min/1.73 m ² , median (IQR)	80 (64, 90.8)	74 (62, 89.3)	88.5 (87.3, 97)	0.02
Blood pressure, mmHg, median (IQR)				
Systolic	123 (114, 129)	120.5 (114, 128.2)	127 (117.2, 130.2)	0.35
Diastolic	71.5 (66.8, 80.3)	70.5 (66, 79.3)	75.5 (69.3, 81.3)	0.61
Mean	83 (75.8, 87.3)	83 (75, 88)	83 (79.3, 87)	0.79
Total cholesterol, mmol/L, median (IQR)	4.4 (3.8, 5.1)	4.4 (3.9, 5.1)	4.5 (3.7, 5.2)	0.93
LDL-C, mmol/L, median (IQR)	2.4 (2, 2.9)	2.3 (2, 2.9)	2.7 (2.1, 2.9)	0.36
HDL-C, mmol/L, median (IQR)	1.1 (0.8, 1.4)	1.1 (0.8, 1.5)	1.1 (0.8, 1.2)	0.42
Triglycerides, mmol/L, median (IQR)	1.4 (1.1, 1.8)	1.4 (1, 1.8)	1.6 (1.3, 1.8)	0.41
Cholesterol ratio, HDL/total cholesterol, median (IQR)	4.4 (3.4, 5)	4.3 (3.3, 5)	4.4 (3.8, 4.6)	0.93
Hs-Troponin T, ng/L, median (IQR)	250 (200, 290.6)	255.5 (210.2, 290)	239.8 (196.7, 301.6)	0.58

Abbreviations: BMI, body mass index; HDL-C, high density lipoprotein; Hs-Troponin T, high sensitivity troponin T; IQR, interquartile range; ISR, in stent restenosis; LDL-C, low density lipoprotein.

^aContinuous and categorical variables compared between outcome groups by Wilcoxon's rank-sum test and chi-square test, respectively.

these patterns remained although without statistical significance. Similarly, sRAGE values at baseline were significantly different between the groups but with patients subsequently developing ISR having lower levels (►Fig. 1B). Like AGE, the AGE/sRAGE ratio was higher both at baseline and at 6 month in ISR subjects compared with those remaining non-ISR (►Fig. 1C). When evaluating the change of these levels within the specific outcome groups (►Fig. 1A–C), the median differences between baseline and 6 months did not meet the Bonferroni-corrected significance.

These serum levels were further explored by stent type (►Fig. 2A–D). Although AGE levels were higher among ISR

subjects, these differences did not reach statistical significance (►Fig. 2A). Patterns were generally similar between the types, with subjects developing ISR having relatively low, unchanging sRAGE levels (►Fig. 2B). DES subjects who remained non-ISR had higher baseline sRAGE levels that remained stable at 6 months. However, BMS subjects remaining non-ISR also had higher sRAGE values than both BMS and DES subjects who developed ISR. These subjects also had an additional statistically significant increase in their sRAGE levels by 6 months. This increase was not seen in the corresponding DES non-ISR subjects, although their baseline sRAGE levels were already frequently higher. The AGE/sRAGE

Table 2 Quantitative coronary angiography characteristics

Variables	All subjects (n = 60)	Non-ISR (n = 48)	ISR (n = 12)	p-Value ^a
Pre-PCI RVD, mm, median (IQR)	3.0	3.0	2.9	0.41
	(2.5, 3.5)	(2.6, 3.5)	(2.5, 3.1)	
Pre-PCI MLD, mm, median (IQR)	2.2	2.1	2.1	0.43
	(1.9, 2.6)	(1.9, 2.6)	(1.9, 2.6)	
Pre-PCI %DS, mm, median (IQR)	0.74	0.75	0.74	0.24
	(0.72, 0.76)	(0.72, 0.76)	(0.7, 0.75)	
Pre-PCI lesion length, mm, median (IQR)	18.6	18.4	19.5	0.02
	(17.9, 19.6)	(17.6, 19)	(18.8, 19.8)	
Post-PCI MLD, mm, median (IQR)	2.2	2.7	2.6	0.40
	(1.9, 2.6)	(2.2, 3.2)	(2.3, 3.0)	
Post-PCI acute gain, mm, median (IQR)	0.51	0.51	0.49	0.68
	(0.39, 0.57)	(0.40, 0.57)	(0.38, 0.56)	
6-month MLD, mm, median (IQR)	2.3	2.6	1.4	<0.0001
	(2, 2.9)	(2.1, 2.9)	(1.2, 1.8)	
Late lumen loss, mm, median (IQR)	0.25	0.23	1.06	<0.0001
	(0.20, 0.38)	(0.19, 0.29)	(1, 1.18)	
Net gain, mm, median (IQR)	0.20	0.25	-0.58	<0.0001
	(0.10, 0.31)	(0.18, 0.33)	(-0.71, -0.49)	

Abbreviations: %DS, percent diameter stenosis; IQR, interquartile range; ISR, in stent restenosis; MLD, mean lumen diameter; Pre-PCI, pre-percutaneous coronary intervention; Post-PCI, immediately post percutaneous coronary intervention; RVD, reference vessel diameter.

^aComparisons made by Wilcoxon rank-sum test.

Table 3 AGE, sRAGE, and MDA levels at baseline to 6 months, overall and by group outcome status

Variables	All subjects (n = 60)	Non-ISR (n = 48)	ISR (n = 12)	p-Value ^a
Pre-PCI AGE, pg/mL, median, (IQR)	2.8×10^7	2.6×10^7	2.9×10^7	0.01
	(2.4×10^7 , 2.9×10^7)	(2.3×10^7 , 2.9×10^7)	(2.8×10^7 , 3.10^7)	
6-month AGE, pg/mL, median, (IQR)	2.6×10^7	2.5×10^7	2.8×10^7	0.07
	(2.3×10^7 , 2.9×10^7)	(2.3×10^7 , 2.9×10^7)	(2.5×10^7 , 3.3×10^7)	
Pre-PCI sRAGE, pg/mL, median (IQR)	690	750	440	<0.0001
	(690, 820)	(650, 890)	(380, 550)	
6-month sRAGE, pg/mL, median (IQR)	790	850	480	<0.0001
	(620, 890)	(750, 930)	(440, 550)	
Pre-PCI AGE/sRAGE ratio, median, (IQR)	4.0×10^4	3.6×10^4	6.8×10^4	<0.0001
	(2.9×10^4 , 5.4×10^4)	(2.7×10^4 , 4.2×10^4)	(5.4×10^4 , 7.9×10^4)	
6-month AGE/sRAGE ratio, median, (IQR)	3.5×10^4	3.10^4	5.7×10^4	<0.0001
	(2.9×10^4 , 4.5×10^4)	(2.6×10^4 , 3.9×10^4)	(4.9×10^4 , 6.6×10^4)	
Pre-PCI MDA, nmol/mL, median (IQR)	1.2	1.1	1.5	<0.0001
	1.01, 1.4	0.98, 1.3	1.4, 1.9	
6-month MDA, nmol/mL, median (IQR)	1.09	1.05	1.56	<0.0001
	0.76, 1.32	0.69, 1.19	1.42, 1.75	

Abbreviations: AGE, advanced glycation end products; IQR, interquartile range; ISR, instent restenosis; MDA, malondialdehyde; Pre-PCI, pre-percutaneous coronary intervention; and sRAGE, soluble receptor for advanced glycation end products.

^aMedian levels compared between non-ISR and ISR groups using Wilcoxon rank sum test.

Fig. 1 Baseline and 6-month post-PCI serum measurements of (A) advanced glycation end products (AGE) levels, (B) soluble receptors for advanced glycation end products (sRAGE) levels, (C) AGE/sRAGE ratios, and (D) malondialdehyde (MDA) levels in non-in-stent restenosis (non-ISR) and in-stent restenosis (ISR) patients. Within the boxplots, dark bars indicate median values, box edges indicate 25th and 75th percentiles, whiskers indicate 1.5 times the interquartile range (IQR; or most extreme value prior), and open dot indicates outlier.

ratio also decreased in these subjects ($\blacktriangleright$ Fig. 2C). In general, AGE/sRAGE ratio levels inversely mirrored sRAGE differences between groups.

Malondialdehyde Levels

Compared with those not developing ISR, baseline serum MDA levels in ISR patients were significantly higher and remained elevated ($\blacktriangleright$ Fig. 1D). The lower MDA levels in non-ISR subjects remained low with a statistically significant 6-month reduction. Within the stent types, baseline MDA levels were higher in subjects who developed ISR compared

with those who did not ($\blacktriangleright$ Fig. 2D). However, at follow-up, DES subjects in both outcome groups demonstrated a decrease in their MDA values from baseline, reaching statistical significance in the non-ISR subjects but not among the few ISR subjects ($n = 4$). As both groups decreased similarly, the 6-month levels remained higher in the ISR versus non-ISR subjects. In contrast, non ISR BMS subjects had no significant change in median MDA levels from baseline to 6 months. The 6-month levels also remained higher in BMS subjects who developed ISR compared with those who did not.

Fig. 2 Baseline and 6-month post-PCI serum measurements of (A) advanced glycation end products (AGE) levels, (B) soluble receptors for advanced glycation end products (sRAGE) levels, (C) AGE/sRAGE ratios, and (D) malondialdehyde (MDA) levels, stratified by stent type (drug-eluting stent [DES] or bare-metal stent [BMS]) and restenosis status (non-in-stent restenosis [non-ISR] or in-stent restenosis [ISR]). Within the boxplots, dark bars indicate median values, box edges indicate 25th and 75th percentiles, whiskers indicate 1.5 times the interquartile range (IQR; or most extreme value prior), and open dot indicates outlier.

Associations between MDA and AGE, sRAGE, and AGE/sRAGE Ratio

The baseline correlation of MDA with AGE is depicted in **Fig. 3A** and at 6 months in **Fig. 3B**. The positive direction at both times (Spearman's correlation values of 0.32 and 0.14, respectively) contrasts the corresponding negative relationship between serum MDA and sRAGE (**Fig. 3C and D**, Spearman's correlation values: -0.39 and -0.32). Lastly, the correlation of serum MDA levels with AGE/sRAGE in ISR patients depicted at baseline (**Fig. 3E**) and at 6 months (**Fig. 3F**) was also positive (Spearman's correlation values of 0.43 and 0.31, respectively).

The 6-month correlations were revisited within BMS and DES patients separately. Spearman's BMS correlation values were 0.22, -0.47 , and 0.48 for AGE, sRAGE, and AGE/sRAGE ratio respectively, while correlation was lost in the DES subjects with corresponding values of 0.04, -0.11 , and 0.05.

Discussion

Higher levels of AGE and AGE/sRAGE ratio as well as lower sRAGE levels were observed pre-PCI among those who subsequently developed ISR over the following 6 months.

Fig. 3 Correlation (r = Spearman's rho) of serum malondialdehyde (MDA) levels with levels of: (1) advanced glycation end products (AGE) at (A) baseline, $r = 0.32$ and (B) 6 months, $r = 0.14$; (2) soluble receptors for advanced glycation end products (sRAGE) levels at (C) baseline, $r = -0.39$, and (D) 6 months, $r = -0.32$; and (3) AGE/sRAGE ratio (E) baseline, $r = 0.43$ and (F) 6 months, $r = 0.31$.

In general, observed levels within the outcome groups did not change during the study period with the exception of sRAGE in BMS patients, where non-ISR subjects displayed a statistically significant increase. Although AGE at both time points differed between subjects who did or did not develop ISR, these differences were markedly more significant when sRAGE levels were accounted for in the AGE/sRAGE ratio. Thus, the negative impact of AGE appears subject to the protective counter-influence of sRAGE. Evaluations of MDA, representing

ROS, demonstrated MDA to be higher at baseline in those who developed ISR. Baseline AGE and AGE/sRAGE positively correlated with ROS (MDA), while sRAGE showed a negative association. A 6-month sRAGE and AGE/sRAGE correlations were similar, although AGE correlation was weaker than at baseline. As **Fig. 2D** suggests that DES stenting may influence MDA levels between assessments independent of AGE and sRAGE, potentially obscuring the physiological relationship between MDA and AGE and/or sRAGE.

Related Literature

The above suggestion that elevated AGE levels and depressed sRAGE levels associate with ISR reflects both animal and human studies to date. In the euglycemic rabbit model of microvascular injury, AGEs aggregate on vessel wall protein and were present in the intima, media, and adventitia.¹⁷ Human studies of vascular segments show that AGEs are increased in regions of atherosclerotic lesions with or without the presence of diabetes.¹⁸ Basta et al demonstrated low pre-PCI sRAGE levels in unstable and stable angina patients who underwent PCI.¹⁹

Our finding of higher MDA levels prior to ISR development is consistent with other research^{17,20} which suggests that the mechanical forces exerted by stenting damages endothelium, resulting in ROS generation. In some patients, this process may trigger a chain reaction culminating in endothelial dysfunction, macrophage activation, smooth muscle cell proliferation, and matrix remodeling (i.e., ISR). Given the observed correlations of AGE and sRAGE with MDA, our data further implicate AGE as contributing to ISR development. This association has biological plausibility as the interaction of AGE with RAGE has been shown to induce ROS generation and nuclear factor kappa B activation.^{4,10,20} Stimulation of nuclear factor kappa B by ROS produces response-to-injury gene transcription, generating cytokines, chemokines, and adhesion molecules that have been implicated in cardiovascular disease.^{3,21,22}

With respect to oxidative stress, our data aligns with reported changes among post-PCI patients that had elevations in oxidative stress postulated to have resulted from plaque destabilization and free radical release from injured endothelial cells during the procedure. However, no significant differences in the incidence of adverse events between stent types were shown and MDA levels were not followed long term.²³ An increased incidence of ISR related to elevated serum, MDA-LDL concentrations, has been observed in diabetic patients.²⁴ Although the patients of the present study are nondiabetic, our finding of rising oxidative stress levels after BMS implantation is consistent with their report. Reports on sRAGE levels in nondiabetics in relation to the type of stent are scarce. Our finding that subjects who developed ISR had lower sRAGE levels at baseline compared with those remaining non-ISR, regardless of stent type, is consistent with our previous report regarding ISR development with BMS implantation.³

Clinical Interpretations

Interestingly, after 6 month the median sRAGE levels had increased among BMS patients without ISR; this change was not observed in any of the other groups and although statistically significant may not relate to a systemic increase induced by the localized stenting event. The lack of a significant serum sRAGE increase in non-ISR DES subjects implies that the local protective release of everolimus may work by a different pathway from systemic sRAGE secretion or that their serum sRAGE levels return to baseline by 6 months. This unexplained difference does not detract; however, from the overall suggestion that baseline sRAGE levels provide an indicator of restenosis likelihood in both stent types.

Changes in MDA levels were found to generally reflect inversion of sRAGE changes. In addition, the AGE/sRAGE ratio inversely mirrored sRAGE differences between groups.

Stratification by stent type similarly showed that oxidative stress at 6 months was higher in subjects who developed ISR compared with those who did not regardless of type. MDA exploration within DES recipients did provide two additional interesting observations: a drop in MDA levels between both ISR and non-ISR subjects at follow-up which was absent in BMS subjects and a higher baseline level among DES patients who developed ISR. The former is potentially due to the sustained effect of everolimus in preventing oxidative stress within the tissues, even though elution from the stent itself is complete by 120 days.²⁵ High baseline MDA levels among those who subsequently developed restenosis even with a DES may be attributable to the action of everolimus in impairing post-PCI re-endothelialization with subsequently decreased nitric oxide bioavailability and vasomotor dysfunction setting the stage for restenosis.¹² Thus prior dysfunctional vascular environments, manifest by high levels of MDA pre-PCI, may be further compromised by the effects of DES placement culminating in ISR. It would appear that in both stent types relatively high baseline values of MDA may be associated with ISR, although thresholds for concern may be higher in DES recipients. The increase in MDA observed after 6 months among the BMS ISR group is not unexpected. It has been suggested that following BMS implantation, the inflammatory response involves the chemotaxis of neutrophils which undergo a respiratory burst with the generation of ROS. BMS deployment also induces the expression of NO_x subunits that increases ROS production.^{21,22} Furthermore, there is abundant evidence that ROS contributes to vasomotor dysfunction, initial apoptosis, proliferation, and migration of vascular smooth muscle and adventitial myofibroblasts, processes that occurs shortly following stent deployment with BMS and precipitate restenosis.^{21,26}

Limitations

There are limitations to these findings. Subjects were not randomized to stent type and as such comparisons between stent types may be confounded by factors that influenced stent choice; however, comparison of DES and BMS subject characteristics did not suggest any overt differences (see supporting information, ►Table S2; available only in the online version). Similarly, generalization of these results must consider the restrictive inclusion criteria, limiting the subjects to middle-aged, nonsmoking, nondiabetic, and Caucasian males. A final limitation of the study is that in our previous study we reported a BMS restenosis rate of 41%. However, in this study we report a BMS restenosis rate of 27%. This difference may be due to other unknowns that could have affected restenosis rates between the two studies such as stent length, vessel size, and location of the stent in the artery. In addition, as sRAGE levels may have affected restenosis rates in this study, we would need to compare the sRAGE levels between the two studies to determine if levels varied.

Future work should evaluate additional groups and specifically design/power to detect difference in AGE, sRAGE, and the AGE/sRAGE ratio in the specified stent types.

Potential Benefits

If further validated, these findings hold potential for at least two major clinical benefits. First, because levels of sRAGE, AGE, and the AGE/sRAGE ratio in the respective outcomes did not significantly change over time, levels at PCI could be utilized to detect patients who require closer follow-up or post-PCI stress testing as they appear at higher risk of ISR. Second, these results suggest that DES stenting may not be necessary in subjects with low risk of ISR based on these biomarkers. Recently, in a randomized PCI trial comparing second generation DES with newer generation BMS, no significant differences were found between those receiving DES compared with those receiving BMS in the composite outcome of death from any cause and nonfatal MI (myocardial infarction).²⁷ Therefore, using BMS may result in a health care savings as these stents have a lower initial cost and do not require prolonged dual antiplatelet therapy.

Conclusion

Irrespective of stent type implanted (DES or BMS), post-PCI restenosis is linked to low serum levels of sRAGE and high serum levels of AGE and AGE/sRAGE ratio pre-PCI. Furthermore, a positive correlation exists between serum levels of AGE and AGE/sRAGE with oxidative stress (MDA) and a negative correlation between serum levels of sRAGE and MDA, suggesting that advanced glycation end product activity may affect known restenosis mechanisms. More extensive studies on the interplay between AGE and sRAGE are needed to evaluate if the serum levels of AGE, sRAGE, or the AGE/sRAGE ratio are useful clinical tools, particularly as early predictors of restenosis risk.

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