Advanced glycation end products impair the functions of saphenous vein but not thoracic artery smooth muscle cells through RAGE/MAPK signalling pathway in diabetes

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Abstract

Saphenous vein (SV) and internal thoracic artery (ITA) are commonly used bypass conduits. However, graft failure occurs in SV rather than in ITA, especially in diabetes (DM). The mechanism for this difference has not been fully understood. Accumulation of advanced glycation end products (AGEs) and activation of AGEs receptor (RAGE) could accelerate smooth muscle cells (SMC) proliferation in DM, we thus asked whether AGEs-RAGE could mediate the differences between SMC from SV (SMCV) and from ITA (SMCA). Twenty-five patients with DM and other 25 patients without DM were enclosed in DM and control group, respectively. AGEs (100 µg/ml) were added to cultured SMCA and SMCV obtained at coronary artery bypass graft (CABG) and proliferative rates were determined. Transcript expression, phosphorylation or protein expression levels of MAP kinase family (ERK, p38 and JNK), matrix metalloproteinases (MMP)-2 and MMP-9 were analysed by real-time PCR, Western-blot or immunofluorescence staining, respectively. Compared with paired SMCA, SMCV showed significantly increased proliferation rate, MAP kinase family phosphorylation, and MMP-2/9 expression in both groups, especially in DM group. The responses of SMCV induced by AGEs were significantly larger in DM than in control group, which could be suppressed by inhibition of RAGE and ERK. However, all the cellular events of SMCV were not found in paired SMCA. This study suggests that AGEs-RAGE could induce the proliferation of SMCV but not SMCA via MAP kinase pathway in DM. It is the intrinsic ‘inactive’ tendency of SMCA that contributes to the different rates of graft disease between SV and ITA after CABG.

Keywords: advanced glycation end products ● RAGE ● mitogen activated protein kinase signalling pathway ● smooth muscle cells ● saphenous vein

Introduction

Saphenous vein (SV) is the most commonly employed grafts in routine coronary artery bypass graft (CABG) procedure [1, 2]. Unfortunately, according to the literature, within 10 years, vein graft failure occurs in nearly half of the conduits [3]. Additionally, patients with diabetic mellitus (DM) tend to have accompanying more advanced, rapidly progressing vein graft disease compared with non-diabetic patients [4–6]. The leading cause of vein graft failure is intimal hyperplasia, which is defined as excessive smooth muscle cells (SMC) proliferation and migration in the intima of SV graft wall [7–9]. It is facilitated by the secretion of growth factors, cytokines and matrix metalloproteinases (MMP) from endothelia and SMC themselves through the activation of key signalling pathways, including mitogen activated protein kinase (MAPK) pathway [10–12]. Recent studies showed that accumulation of advanced glycation end products (AGEs) and activation of receptor of AGEs (RAGE) were found to accelerate the vascular remodelling in diabetic patients [13–15]. Therefore, in this study, we chose to investigate the proliferation and MMP-2, MMP-9 expression of SMC from SV induced by AGEs-RAGE via MAPK signalling pathway in diabetic patients.

On the other hand, internal thoracic arteries (ITA) are another commonly used graft in coronary surgical practice. As well known, ITA grafts show the overwhelming superiority to SV grafts due to

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their long-term patency rates [16]. However, until now, the mechanism for the inherent function differences between venous and arterial SMC has not been fully elucidated. Thus, in the present study, we also explored whether AGEs-RAGE/MAPK signalling pathway could induce different reactions in paired SMC from SV (SMCV) and ITA (SMCA) under controlled conditions.

Material and methods

Study participants

The study was approved by the local Research Ethics Committee, and each patient gave written informed consent. Twenty-five individuals with type 2 DM had fasting blood glucose >5.5 mmol/l and/or current treatment with insulin or oral hypoglycaemic agents. The non-diabetic (control) group comprised the other 25 patients who had no pre-operative DM history. Patients previously diagnosed as hyperlipidaemia (total cholesterol level >200 mg/dl) or hypertensive (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg) were on appropriate lipid-lowering or hypotensive agent treatments. Individual characteristics of the patients are presented in Table 1.

SMC culture

Samples of SV and ITA were obtained at the time of CABG operation. Parts of the grafts were minced and cultured in DMEM contained 10% foetal calf serum (FCS) until SMC came out. The cells were then maintained in DMEM containing 10% FCS in a humidified 5% CO2/95% air atmosphere at 37°C.

Preparation of AGEs

Advanced glycation end products were prepared as previously reported [17]. Briefly, bovine serum albumin (BSA) was incubated with 0.5 mol/l glucose in PBS in the dark for 16 weeks at 37°C in the presence of 1.5 mmol/l phenylmethylsulphonyl fluoride, 0.5 mmol/l ethylenediaminetetraacetic acid, penicillin (100 U/ml) and streptomycin (100 U/ml) under sterile conditions. Control non-glycated BSA was incubated in the absence of glucose under the same conditions. The concentration of the AGEs-BSA solution used in this study was 100 μg/ml. The AGEs-BSA solution was confirmed to be endotoxin free (<0.5 U/ml of endotoxin).

SMC proliferation assays

To assess the different pre-existing proliferative property, paired SMCV and SMCA were seeded in parallel into 24-well tissue culture plates at a density of 1 × 10⁴ cells per well in full growth medium (DMEM plus 10% FCS). The medium was replaced every 2 days. After 5 days, the cells were released from culture wells and cell viability was monitored by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cell were seeded onto 96-well plates (1 × 10⁴ cells/well). After treatment, MTT tetrazolium salt (Sigma-Aldrich, St. Louis, MO, USA., 0.2 mg/ml) was added to each well, cells were further incubated in 5% CO2 at 37°C for 4 hrs. DMSO was added to dissolve formazan crystals for 20 min. The number of viable cells was assessed by measurement of the absorbance at 490 nm using a Safire 2 microplate reader (TECAN, San Jose, CA, USA).

To study the effect of AGEs on SMC proliferation, cells were seeded at 1 × 10⁴ cells per well. After 24 hrs, the full growth medium was changed, and the cells were incubated with fresh medium containing AGEs-BSA (100 μg/ml) for 12 hrs or pre-treated with anti-RAGE

### Table 1 The Demographics of the Studied Population

|                          | Control (n = 25) | DM group (n = 25) | P-value |
|--------------------------|-----------------|------------------|---------|
| Gender (male/%)          | 20 (80.0)       | 22 (88.0)        | 0.18    |
| Age (year)               | 62.8 ± 10.5     | 63.2 ± 11.8      | 0.53    |
| Bodyweight (kg)          | 66.3 ± 14.7     | 64.7 ± 11.3      | 0.46    |
| Smoking (n%)             | 11 (44.0)       | 9 (36.0)         | 0.07    |
| Hypertension (n%)        | 12 (48.0)       | 14 (56.0)        | 0.09    |
| Hyperlipidaemia (n%)     | 8 (32.0)        | 9 (36.0)         | 0.11    |
| Cerebral vessel disease (n%) | 9 (36.0)  | 10 (40.0)        | 0.14    |
| Peripheray vessel disease (n%) | 6 (24.0) | 7 (28.0)         | 0.10    |
| Chronic renal failure (n%) | 3 (12.0)     | 4 (16.0)         | 0.08    |
| Chronic obstructive pulmonary disease (n%) | 4 (16.0) | 3 (12.0)        | 0.08    |
| Diabetic history (year)  | ...             | 10.5 ± 4.7       | ...     |
| Fasting blood glucose (mmol/l) | 5.2 ± 1.1 | 14.17 ± 5.63    | <0.01   |
antibodies (20 μg/ml) for 1 hr and then incubated with AGEs-BSA (100 μg/ml) for 12 hrs. and then full growth medium was changed, the cells were continued to culture for 24 hrs.

Real-time PCR

Real-time PCR was performed with an Applied Biosystems 7300 Real-time PCR System with TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). The amplification cycle consisted of 2 min. at 50°C, 10 min. at 95°C, 15 sec. at 95°C, and 1 min. at 60°C. GAPDH served as controls for PCR. Relative gene expression levels were quantified using the 2^(-ΔΔCt) formula.

Western Blot analysis

Total proteins isolated from vessels or culture SMC were size-fractionated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore, Billerica, MA, USA). Each membrane was incubated with specific primary antibodies (Calbiochem, San Diego, CA, USA) and subsequently with secondary antibody (Jackson Immunolab, West Grove, PA, USA). GAPDH or β-actin served as the internal controls. Immune complexes were visualized with the enhanced chemiluminescence detection system (Amersham, Piscataway, NJ, USA). Quantification of bands was performed by densitometry using the LAS-3000 Imaging System (FUJIFILM, Kanagawa, Japan) and NIH Image J software.

Immunofluorescent stain analysis

Serial 3-μm paraffin sections of paired ITA and SV were dewaxed and rehydrated. After the sections were blocked, sections were incubated for 1 hr at room temperature with primary antibodies (Santa Cruz Biotechnology Inc., Dallas, TX, USA) diluted in 1.5% BSA in PBS. Paired SMCa and SMCv were washed by PBS, fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After the cells were incubated with BSA for 30 min., the primary antibodies were added overnight at 4°C. The sections and SMC were rinsed, incubated with Fluorescein isothiocyanat (FITC)-conjugated (Invitrogen, Carlsbad, CA, USA) or Cy3-conjugated (Jackson ImmunoResearch) secondary antibodies for 1 hr at room temperature in the dark room, and then counterstained with 4',6'-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). The fluorescent signals were observed under the fluorescence microscope (Zeiss, Munich, Germany).

Statistical analysis

All values were expressed as means ± S.D. Paired and/or unpaired Student’s t-tests were used as appropriate to evaluate the statistical significance of differences between two group means, and ANOVA was performed for multiple groups by one-way ANOVA. *P < 0.05 was considered statistically significant. Statistical analysis was performed with SAS 6.12 software (SAS Institute Inc, Chicago, IL, USA).

Results

Demographic characteristics of patients

The demographics of the studied population did not differ in the two groups except for parameters related to diabetic history. The two groups were well-matched for age, gender and presentation profile (Table 1).

AGEs has different effects on proliferation of SMCv and SMCa

Compared with paired SMCa, SMCv showed increased proliferative property in both of the two groups (Fig. 1, blank). And the difference of the inherent proliferative property between paired SMCv and SMCa was more noticeable in DM group than the control group (P < 0.05 and <0.01 in control and DM group, respectively), indicating that SMCv in DM group showed more significantly increased proliferation than SMCv in control group. Interestingly, there was no difference of proliferation in SMCa between the two groups (P > 0.05). In the stimulatory study, it was observed that the SMCv treated with AGEs-BSA showed significantly increased proliferation than paired SMCa in both groups (Fig. 1, AGEs, P < 0.01), and the increase in DM group was significantly larger than in control group (P < 0.01). However, the proliferative increase of SMCa induced by AGEs-BSA in the two groups did not reach statistical significance (P > 0.05). Furthermore,
pre-treating cells with RAGE-neutralizing antibody could attenuate AGEs-induced proliferative increases of SMCV in both groups. These results indicate that AGEs-RAGE can induce the increase in the proliferation of SMCV but not SMCA, and the increase is more significant in patients with DM than those without DM.

Transcript expression levels of MAPK family and MMPs mRNA in SMC induced by AGEs

Mitogen activated protein kinase pathway is important for cells to respond to numerous growth factors. Matrix metalloproteinases have the effects on promoting the migration of SMC. We therefore examined the gene expression of these molecules (Fig. 2). Compared with paired SMCA, SMCV showed increased transcript expression of MAPK family (ERK, p38, and JNK), MMP-2 and MMP-9 mRNA in both of the two groups, and the difference of the expression between paired SMCV and SMCA was more noticeable in DM group than the control group (Fig. 2, \( P < 0.05 \) and \( < 0.01 \) in control and DM group, respectively), indicating that SMCV in DM group showed more significantly increased proliferation than SMCA in control group. However, there was no difference of the expression in SMCA between the two groups (\( P > 0.05 \)). After stimulation with AGEs, transcript expression levels of the MAPK family and MMP-2/9 gene were further increased in SMCV of the two groups (Fig. 2, SMCV plus AGES versus that without AGES, \( P < 0.05 \) and \( P < 0.01 \) in control and DM, respectively). And this phenomenon was more notable in DM group (\( P < 0.01 \) versus control group). In contrast with these changes in SMCV, the relative transcript expressions of these genes had no significant difference in SMCA from both groups (\( P > 0.05 \)). RAGE antibody treatment could reduce transcript expression levels of MAPK family and MMPs mRNA in SMCV compared to that of the untreated SMCV, \( P < 0.05 \), respectively. However, it was not found in SMCA of the two groups, \( P > 0.05 \), respectively. These data suggest that AGEs-RAGE can elevate the transcript expression levels of MAPK family and MMPs mRNA in SMCV, but not in SMCA, especially in DM group.

Phosphorylation of MAPK family and expression of MMPs proteins in SMC

To further investigate the different reactions of paired SMCV and SMCA induced by AGEs, we examined the phosphorylation levels of various MAPK family proteins and expression levels of MMP-2/9 proteins in SMC by Western blotting. Compared with paired SMCA, SMCV showed increased phosphorylation levels of ERK, p-38 and JNK and protein expression levels of MMP-2/9 in both of the two groups (Fig. 3A and B, p-ERK, p-p38, p-JNK). And the difference in the
phosphorylation and the protein expression between paired SMCV and SMCA was bigger in DM group than the control group (Fig. 3B, \( P < 0.05 \) and \( P < 0.01 \) in control and DM, respectively; DM versus control, \( P < 0.05 \)). Interestingly, there was no difference in the phosphorylation or expression of these proteins in SMCA between the two groups (\( P > 0.05 \)). Moreover, after stimulation with AGEs, compared with SMCV without the stimulation, the levels of p-ERK, p-p38, p-JNK, MMP-2 and MMP-9 proteins were significantly more increased in SMCV but not in SMCA, especially in DM group.

MAPK family is involved in AGEs-induced expression of MMPs in SMCV

To ask whether AGEs-RAGE induces the expression of MMPs in SMCV through MAPK family, we used an inhibitor of ERK, PD98059 to pre-treat the cells. Pre-treating with PD98059 significantly abrogated AGEs-induced expression of MMP-2 and MMP-9 in SMCV from both of the two groups (Fig. 5A and B, \( P < 0.05 \) or \( P < 0.01 \), respectively). The data suggest that AGEs stimulates the expression of MMP-2 and MMP-9 proteins through MAPK family in SMCV, but not in SMCA.

Coexpression of RAGE and MMPs in SMC

To further confirm the above findings in vitro, we finally tested the coexpression of RAGE and MMPs in paired SV and ITA in vivo and SMCV and SMCA in vitro by immunofluorescent staining. The results revealed that compared with the paired ITA or SMCA, the fluorescent signals labelled with RAGE (FITC), MMP-2 and -9 (Cy3) were significantly increased in the media of SV or SMCV in both groups, especially in DM group (Fig. 6A and B). However, the fluorescent signals of these proteins had no significant difference in the media of ITA or SMCA in both groups (Fig. 6A and B). Double labelled immunofluorescent results distinctly confirmed that the relations between protein levels of RAGE and MMPs had significant difference in the media between paired SV and ITA conduits or paired SMCV and SMCA.

Discussion

Saphenous vein is the most commonly used conduit in CABG procedure, although it is prone to post-operative graft disease [1, 2]. Many studies have attempted to elucidate the mechanism of vein graft disease [18–20]. Intima hyperplasia serves as the foundation for subsequent progressive graft atheroma which eventually results in occlusion of the SV grafts years after CABG [21, 22]. Abnormal proliferation and migration of SMC from the media to the intima through internal elastic lamina are key events in the development of intima hyperplasia [23]. Before migration of SMC, the surrounding extracellular matrix must be initially degraded by the matrix metalloproteinases MMPs [24]. Of several MMPs, MMP-2, MMP-9 possess the unique ability to degrade elastin and collagen, the main components of the basilar membrane [25]. However, the relation between MMPs in SMC and SV graft disease remained unknown. We here clearly demonstrate precise molecular and cellular mechanisms involved in the native pathological remodelling of
Fig. 4 The effect of AGEs on the protein levels of MAPK family and MMP-2, MMP-9 in paired SMC. The protein phosphorylation or expression levels of MAPK family and MMPs were analysed by Western blotting in paired SMC<sub>A</sub> and SMC<sub>V</sub> obtained from patients with (DM) or without (control, Ctr) DM. (A) Representative images are shown. GAPDH served as an internal control. (B and C) Statistical analysis for the expression levels of p-ERK, ERK, p-p38, p38, p-JNK, JNK, MMP-2 and MMP-9, respectively. Values are shown as the ratio to GAPDH expression. AGEs<sup>+</sup> means SMC incubated with fresh medium containing AGEs-BSA (100 μg/ml) for 12 hrs anti-RAGE<sup>+</sup> means SMC neutralized with anti-RAGE antibodies (20 μg/ml) for 1 hr and then incubated with AGEs-BSA (100 μg/ml) for 12 hrs. Data are expressed as means ± S.D. (N = 8). ** versus #, P < 0.05; ** versus ##, P < 0.01; ## versus #, P < 0.01.
SV in diabetic patients characterized by SMC proliferation and MMPs over-expression.

The metabolic effects of hyperglycaemia in diabetic patients may render them vulnerable to vascular complications [3, 26]. However, even when blood glucose is well controlled, intimal hyperplasia is more prevalent in vein grafts of diabetic patients, suggesting that additional factors contribute to their poor outcome [27]. Some studies suggest that AGEs accelerate atherosclerosis in diabetic patients with coronary heart disease [13–15]. Advanced glycation end product-induced cell autophagy has been shown to contribute to the process of proliferation of Vascular Smooth Muscle Cells (VSMCs), which is related to atherosclerosis in diabetes [15]. Advanced glycation end products may also induce calcification of VSMCs by osteoblast-like differentiation of the cells through RAGE/p38 MAPK signalling pathway [28]. Moreover, proliferation of SMC, a key factor in the development of atherosclerotic lesions, is significantly stimulated by the accumulation of AGEs and their interaction with RAGE [29]. Activation of RAGE not only accelerates early lesion formation but sustains lesion progression in the diabetic apoE-null mouse model [30]. RAGE activation in VSMC could induce the development of vascular diseases by interfering with the contractile phenotype of VSMCs through RAGE/p38 MAPK signalling pathway [28]. Moreover, proliferation of SMC, a key factor in the development of atherosclerotic lesions, is significantly stimulated by the accumulation of AGEs and their interaction with RAGE [29]. Activation of RAGE not only accelerates early lesion formation but sustains lesion progression in the diabetic apoE-null mouse model [30]. RAGE activation in VSMC could induce the development of vascular diseases by interfering with the contractile phenotype of VSMCs through RAGE/p38 MAPK signalling pathway [28].

On the other hand, numerous studies have shown the superiority of ITA in promoting long-term survival and reduced recurrence of major adverse cardiac events after CABG in all patients, including those with DM [16, 32]. Some studies about why graft disease is more prevalent in SV than ITA have focused on the effects from surgical trauma during operative manipulation and altered hemodynamic conditions [33, 34]. However, theory is growing that SMCV and SMCA are derived from different embryonic origins and exhibit distinct functions which may contribute to the different rate of graft disease observed in SV versus ITA grafts [34]. This raises the question of whether SMCV is intrinsically more 'active' than paired SMCA.

Some studies have revealed that AGEs have been shown to induce proliferation SMC, increase generation of reactive oxygen species, decrease nitric oxide bioavailability and up-regulate the production of various cytokines or growth factors, such as tumour necrosis factor-α, platelet-derived growth factor and Vascular Cell Adhesion Molecule 1 (VCAM-1) [35, 36]. Human SV-SMCs are inherently more proliferative and invasive than paired IMA-SMCs, likely due to a relative increase in p44/42-MAPK activation [23]. However, even now, there is no direct evidence for the reaction in SMCA induced by AGEs-RAGE through MAPK signalling pathway in diabetic conditions. Therefore, we explored whether AGEs-RAGE/MAPK signalling pathway could induce different reactions in paired SMCV and SMCA under controlled conditions. In this study, the activation of SMCV induced by AGEs-RAGE/MAPK signalling pathway was not found in SMCA from any subgroups in our study. SMCA exhibited much lower inherent proliferation, MAPK family protein phosphorylation and MMPs secretion

![Fig. 5](image-url) The role of ERKs in AGE-induced expression of MMP-2 and MMP-9 in paired SMC. Paired SMC obtained from patients with DM (DM) or without control (Ctr) were pre-treated with the ERK inhibitor PD98059 (20 μM) (+) or vehicle (-) for 30 min. and then incubated with AGES-BSA (100 μg/ml) for 12 hrs. The expression of MMP-2 and MMP-9 protein was analysed by Western blotting. (A) Representative images are shown. β-actin served as an internal control. (B) Quantitative analysis of MMP-2 and MMP-9 expressions. Values represent the ratio to β-actin expression. Data are expressed as means ± S.D. (N = 8). ** versus #, P < 0.01; ** versus ##, P < 0.01; #** versus #, P < 0.01.
Coexpression of RAGE and MMPs in paired ITA and SV and paired SMCa and SMCv. Paired ITA and SV and paired SMCa and SMCv obtained from patients with (DM) or without (control, Ctr) DM were doubly labelled with anti RAGE and MMP-2 or anti RAGE and MMP-9 antibodies, respectively, by immunofluorescent staining. Respective two sets of representative photos of paired ITA and SV and paired SMCa and SMCv from eight experiments are shown. Green and red fluorescence indicate RAGE and MMP-2/9 signals, respectively. Cell nuclear are stained by DAPI. (A) Paired ITA and SV. (B) Paired SMCa and SMCv. Original magnification ×100.
Fig. 6 Continued.
compared with paired SMCv in both diabetic and non-diabetic subgroups. It is the intrinsic ‘inactive’ tendency of SMCv that may contribute to the obviously different rates of graft disease between SV and ITA after coronary surgery.

PI3K/Akt signalling pathway, particular the p-Akt is required to test for elucidating the mechanism of SMC proliferation. We also examined the expression of PI3K/p-PI3K and Akt/p-Akt. The results show that there are no significant differences in the activation of PI3K/Akt after stimulation with AGE between paired SMCv and SMCv (Fig. S1), suggesting that PI3K/Akt signalling is not involved in the mechanisms underlying the different graft rate failure between SV and ITA grafts. The precise reason for why MAPK family, but not Akt, contributes to AGEs-induced MMPs secretion and SMCv proliferation in DM remains to further study.

Nevertheless, these observations raise the question of whether a local genetic treatment should be considered in diabetic patients with venous bypass grafts. Novel therapeutic approaches may develop that transform the SV conduit into a vessel with an increased long-term patency. We hope our study may provide novel information for further understanding graft failure in diabetic patients after CABG procedure.

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### Conflicts of interest

The authors confirm that there are no conflicts of interest.

### Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** The effect of AGEs on the phosphorylation and expression levels of PI3K and AKT proteins in paired SMC.

### References

1. Motwani JG, Topol EJ. Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. *Circulation*. 1998; 97: 916–31.
2. Angelini GD, Newby AC. The future of saphenous vein as a coronary artery bypass conduit. *Eur Heart J*. 1989; 10: 273–80.
3. Campeau L, Enjalbert M, Lesperance J, et al. Atherosclerosis and late closure of aortocoronary saphenous vein grafts: sequential angiographic studies at 2 weeks, 1 year, 5 to 7 years, and 10 to 12 years after surgery. *Circulation*. 1983; 68(3 Pt 2): I11–7.
4. Niccoli F, Agostinelli A, Vezzani A, et al. CAVB versus PCI in the treatment of diabetic patients affected by coronary artery disease which is the best option? *Int Heart J*. 2014; 55: 469–73.
5. Deb S, Singh SK, Moussa F, et al. The long-term impact of diabetes on graft patency after coronary artery bypass grafting surgery: a substudy of the multicenter radial artery patency study. *J Thorac Cardiovasc Surg*. 2014; 148: 1246–53.
6. Kapur A, Bartolini D, Finlay MC, et al. The bypass angioplasty revascularization in type 1 and type 2 diabetes study: 5-year follow-up of revascularization with percutaneous coronary intervention versus coronary artery bypass grafting in diabetic patients with multivessel disease. *J Cardiovasc Med (Hagerstown)*. 2010; 11: 26–33.
7. Kishikawa M, Lopes RD, Reyes EM, et al. Long-term clinical and angiographic outcomes in patients with diabetes undergoing coronary artery bypass graft surgery: results from the project of ex-vivo vein graft engineering via transfection IV trial. *Am Heart J*. 2015; 169: 175–84.
8. Sur S, Sugimoto JT, Agrawal DK. Coronary artery bypass graft: why is the saphenous vein prone to intimal hyperplasia? *Can J Physiol Pharmacol*. 2014; 92: 531–45.
9. Riches K, Alishanwani AR, Warburton P, et al. Elevated expression levels of miR-143/5 in saphenous vein smooth muscle cells from patients with Type 2 diabetes drive persistent changes in phenotype and function. *J Mol Cell Cardiol*. 2014; 74: 240–50.
10. Kallenbach K, Salcher R, Heim A, et al. Inhibition of smooth muscle cell migration and neointima formation in vein grafts by overexpression of matrix metalloproteinase-3. *J Vasc Surg*. 2009; 49: 750–8.
11. Perek B, Malinka S, Miisterski M, et al. Preexisting high expression of matrix metalloproteinase-2 in tunica media of saphenous vein conduits is associated with unfavorable long-term outcomes after coronary artery bypass grafting. *Biomed Res Int*. 2013; 2013: 703721.
12. Muto A, Panchal A, Kim N, et al. Inhibition of mitogen activated protein kinase activated protein kinase II with MML-0100 reduces intimal hyperplasia ex vivo and in vivo. *Vasc Pharmacol*. 2012; 56: 47–55.
13. Barlovic DP, Soro-Paavonen A, Jandeleit-Dahm KA. RAGE biology, atherosclerosis and diabetes. *Clin Sci*. 2012; 121: 43–55.
14. Torreggiani M, Liu H, Wu J, et al. Advanced glycation end product receptor-1 transgenic mice are resistant to inflammation, oxidative stress, and post-injury intimal hyperplasia. *Am J Pathol*. 2009; 175: 1722–32.
15. Hu P, Lai D, Lu P, et al. ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells. *Int J Mol Med*. 2012; 29: 613–8.
16. Schwann TA, Tranbaugh RF, Dimitrova KR, et al. Time-varying survival benefit of radial artery versus vein grafting: a multistitutional analysis. *Ann Thorac Surg*. 2014; 97: 1328–34.
17. Hou FF, Chertow GM, Kay J, et al. Interaction between beta 2-microglobulin and advanced glycation end products in the development of dialysis-related amyloidosis. *Kidney Int*. 1997; 51: 1514–9.
18. Khaleel MS, Dorchheim TA, Duryee MJ, et al. High-pressure distention of the saphenous vein during preparation results in increased markers of inflammation: a potential mechanism for graft failure. *Ann Thorac Surg*. 2012; 93: 552–8.
19. Chello M, Spadaccio C, Lusini M, et al. Advanced glycation end products in diabetic
patients with optimized glycaemic control and their effects on endothelial reactivity: possible implications in venous graft failure. Diabetes Metab Res Rev. 2009; 25: 420–6.

20. Hilker M, Buerke M, Lehr HA, et al. Bypass graft disease: analysis of proliferative activity in human aorto-coronary bypass grafts. Heart Surg Forum. 2002; 5 (Suppl. 4): S331–41.

21. Mitra AK, Gangahar DM, Agrawal DK. Cellular, molecular and immunological mechanisms in the pathophysiology of vein graft intimal hyperplasia. Immunol Cell Biol. 2006; 84: 115–24.

22. Schachner T, Steger C, Heiss S, et al. Paclitaxel treatment reduces neointimal hyperplasia in cultured human saphenous veins. Eur J Cardiothorac Surg. 2007; 32: 906–11.

23. Turner NA, Ho S, Warburton P, et al. Smooth muscle cells cultured from human saphenous vein exhibit increased proliferation, invasion, and mitogen-activated protein kinase activation in vitro compared with paired internal mammary artery cells. J Vasc Surg. 2007; 45: 1022–8.

24. Guzeloglu M, Reel B, Atmaca S, et al. The effects of PPARγ agonist rosiglitazone on neointimal hyperplasia in rabbit carotid anastomosis model. J Cardiothorac Surg. 2012; 7: 57.

25. Lee T, Esemuede N, Sumpio BE, et al. Thrombospondin-1 induces matrix metalloproteinase-2 activation in vascular smooth muscle cells. J Vasc Surg. 2003; 38: 147–54.

26. Dwivedi A, Sala-Newby GB, George SJ. Regulation of cell-matrix contacts and beta-catenin signaling in VSMC by integrin-linked kinase: implications for intimal thickening. Basic Res Cardiol. 2008; 103: 244–56.

27. Sun YX, Lin ZB, Ding WJ, et al. Preoperative glucose level has different effects on the endogenous extracellular matrix-related gene expression in saphenous vein of type 2 diabetic patients undergoing coronary surgery. Diab Vasc Dis Res. 2014; 11: 226–34.

28. Tanikawa T, Okada Y, Tanikawa R, et al. Advanced glycation end products induce calcification of vascular smooth muscle cells through RAGE/p38 MAPK. J Vasc Res. 2009; 46: 572–80.

29. Nasib E, Tomoya M, Eisuke Y, et al. Anergilipin, a DPP-4 inhibitor, suppresses proliferation of vascular smooth muscles and monocyte inflammatory reaction and attenuates atherosclerosis in male apo E-deficient mice. Endocrinology; 2013; 154: 1263–70.

30. Buccinelli LG, Wendt T, Qu W, et al. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. Circulation. 2002; 106: 2827–35.

31. Elie Simard, Soeliradi T, Maltais JS, et al. Receptor for advanced glycation end-products signaling interferes with the vascular smooth muscle cell contractile phenotype and function. PLoS ONE. 2015; 10: e0128881.

32. Nwasokwa ON. Coronary artery bypass graft disease. Ann Intern Med. 1995; 123: 528–45.

33. Sarah JG, Alla BZ, Andrew CN. Surgical preparative injury and neointima formation increase MMP-9 expression and MMP-2 activation in human saphenous vein. Cardiovasc Res. 1997; 33: 447–59.

34. Riches K, Warburton P, O’Regan DJ, et al. Type 2 diabetes impairs venous, but not arterial smooth muscle cell function: possible role of differential RhoA activity. Cardiovasc Revasc Med. 2014; 15: 141–8.

35. Jia G, Cheng G, Gangahar DM, et al. Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. Immunol Cell Biol. 2008; 86: 448–54.

36. Correa-Giannella ML, de Azevedo MR, Lerohl D, et al. Fibronectin glycation increases IGF-1 induced proliferation of human aortic smooth muscle cells. Diabetol Metab Syndr. 2012; 4: 19.