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Letters

Dipeptidyl Peptidase-4 Inhibition Prevents Vascular Calcification by Potentiating the Insulin-Like Growth Factor-1 Signaling Pathway

Cardiovascular calcification is a growing burden and a leading contributor to acute cardiovascular events, but no therapeutics are currently available. Recently, Choi et al. (1) put forward dipeptidyl peptidase (DPP)-4 as a new pharmacological target to prevent aortic valve calcification. DPP-4 is an exopeptidase that cleaves many substrates, including insulin-like growth factor (IGF)-1 to a less bioactive molecule at the IGF-1 receptor, which is suggested to induce a potent anti-calcifying effect (2) However, as previously stressed (3), it remains to be carefully investigated whether DPP-4 inhibition represents a new strategy to prevent cardiovascular calcification and to ascertain if this anti-calcifying effect is related to the prevention of DPP-4–mediated IGF-1 inactivation.

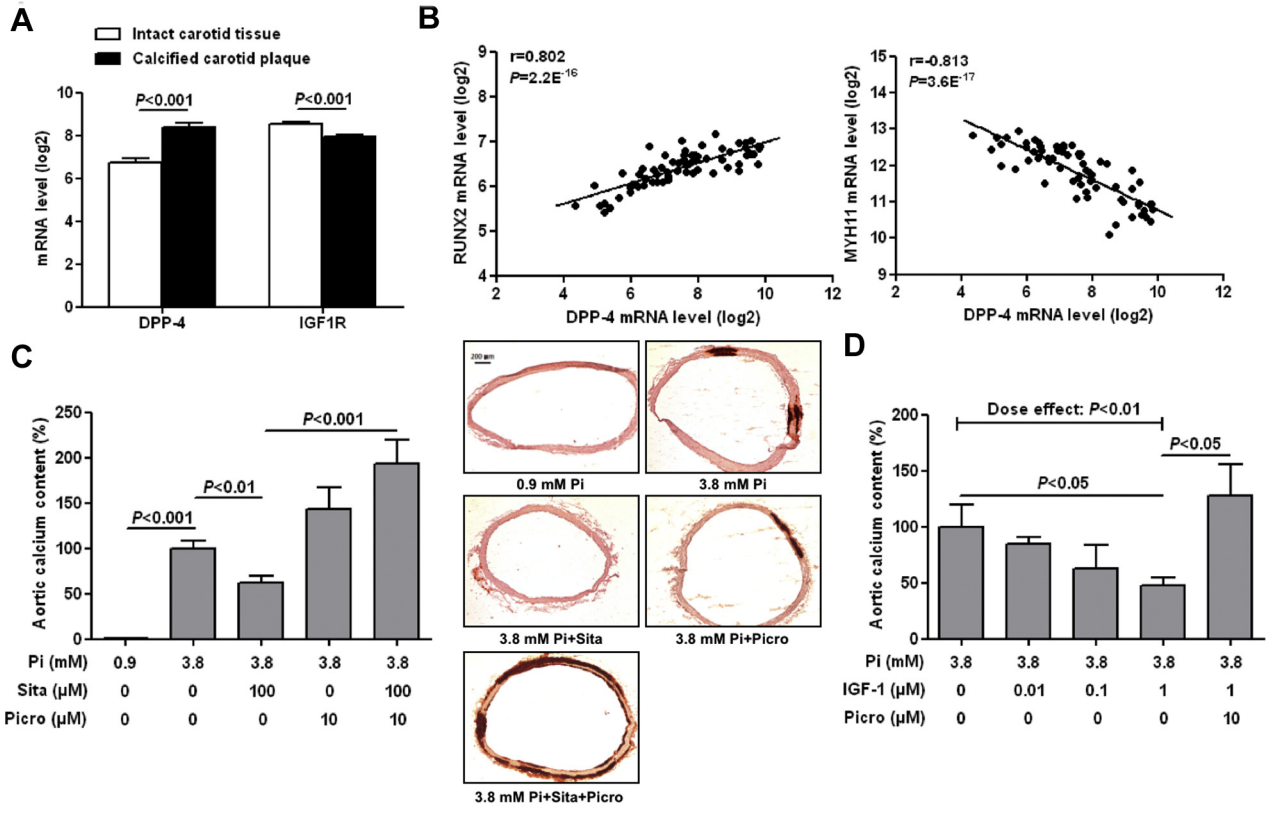
For this objective, we compared the global gene expression profiles of human calcified carotid atherosclerotic plaques with those of adjacent sites, obtained from endarterectomy specimens (4) using Affymetrix GeneChip Human Gene 1.0 ST arrays (Affymetrix, Santa Clara, California). We assessed the mRNA expression level of DPP-4 and its relation with mRNA levels of the osteochondogenic and contractile markers, Runt-related transcription factor 2 (RUNX2) and myosin heavy chain 11 (MYH11), respectively. Furthermore, we assessed the impact of the DPP-4 inhibitor, sitagliptin, alone or combined with picropodophyllin, which inhibits both the activity of the IGF-1 receptor and its expression, on vascular calcification. We used an ex vivo mineralization assay of the aorta isolated from male wild-type Wistar rats and cut into 2- to 3-mm rings (3 to 5 rings per aorta). Calcium content of rat aortic rings was determined colorimetrically with o-cresolphthalein complexone.

Differences between the mRNA expression levels of DPP-4 and the IGF-1 receptor between human calcified carotid plaques and adjacent sites were assessed by paired *t* test corrected for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) procedure. Pearson's correlation analyses were used to assess the relationships between DPP-4 and RUNX2 and MYH11 mRNA expression levels. To consider the variability in the degree of calcification between isolated rat aortas, differences between groups for the calcium content of rat aortic rings were analyzed using a generalized linear model with group as a factor and the aorta used as a co-factor, followed by Tukey-Kramer multiple comparison tests for post hoc analysis. A p value of <0.05 was considered statistically significant.

Similar to the results obtained for the human aortic valve (1), DPP-4 was among the most upregulated genes in calcified carotid plaques compared with adjacent sites (3.21-fold; FDR <0.001) (**Figure 1A**). Moreover, we observed a strong positive correlation between DPP-4 and RUNX2 gene expression levels and an inverse relation between DPP-4 and MYH11 expression levels (**Figure 1B**). Interestingly, calcified carotid plaques were also characterized by a marked decrease in IGF-1 receptor gene expression compared with adjacent sites (0.66-fold; FDR <0.001) (**Figure 1A**). These results supported a role for DPP-4 and IGF-1 in the vascular calcification process.

Culture of rat aortic rings in high-phosphate conditions (3.8-mM inorganic phosphate) during 7 days induced an increase in the aortic calcium content that was reduced by the addition of the DPP-4 inhibitor sitagliptin to the culture medium, as illustrated by calcium deposition with alizarin red staining (**Figure 1C**) and by increasing concentrations of IGF-1 (**Figure 1D**). The addition of the IGF-1 receptor inhibitor picropodophyllin prevented the inhibitory effect of sitagliptin (**Figure 1C**) and exogenous IGF-1 (**Figure 1D**) on aortic calcification, which supported the concept that the vascular anti-calcifying effect of DPP-4 inhibition was related to the potentiation of IGF-1 signaling. In a second set of experiments, we showed that the aortic calcification induced by high-phosphate conditions was significantly enhanced by the combination of sitagliptin and picropodophyllin (32.1 ± 7.1 μg/mg vs. 140.8 ± 7.1 μg/mg; $n = 4$ per condition; $p < 0.05$), but was unchanged in the

FIGURE 1 Role of the DPP-4/IGF-1 Signaling Pathway in Vascular Calcification



(A) mRNA expression levels of dipeptidyl peptidase-4 (DPP-4) and the insulin growth factor-1 (IGF-1) receptor between human calcified carotid plaques (n = 34) and distant intact tissues (n = 34). **(B)** Relationships between mRNA expression levels of DPP-4 with mRNA levels of runt-related transcription factor 2 (RUNX2) and myosin heavy chain 11 (MYH11). **(C)** Calcium content and representative images of calcium accumulation with Alizarin red staining in rat aortic rings cultured in normal (0.9-mM inorganic phosphate [Pi]) and high-phosphate (3.8-mM Pi) conditions during 7 days in the absence and in the presence of the DPP-4 inhibitor sitagliptin (Sita) and/or the inhibitor of IGF-1 receptor picropodophyllin (Picro) (n = 6 to 9 per condition). **(D)** Calcium content of rat aortic rings cultured in 3.8-mM Pi conditions during 7 days in the presence of increasing concentrations of IGF-1 and Picro (n = 4 per condition). Data are mean ± SEM.

presence of sitagliptin associated with the dual inhibitor of the IGF-1 and insulin receptors, BMS-754807 (40.5 ± 15.2 μg/mg; n = 4; $p < 0.05$ vs. sitagliptin and picropodophyllin). This suggested that the activation of the insulin receptor by IGF-1, which becomes prominent during blockade of the IGF-1 receptor, contributed to vascular calcification (5).

Ours results showed that alteration of DPP-4 and the IGF-1 axis represents a new mechanism of vascular calcification. Inhibitors of DPP-4 represent an exciting pharmacological avenue to slow vascular calcification by preventing IGF-1 inactivation and restoring IGF-1 receptor-dependent signaling. However, as previously stressed (2), IGF-1 was also shown to potentiate osteoblastic bone formation; inhibition of DPP-4 could be rather detrimental at an advanced stage of the calcific disease. In addition, DPP-4 metabolizes many other substrates that could exert unintended adverse effects in vivo, in particular, on cardiovascular calcification. Additional experiments are thus needed to assess the impact of DPP-4 inhibitors at this level in humans, but the cumulative experimental evidence strongly suggests that they may help to finally prevent cardiovascular calcification and its associated complications.

Olivier Varennes, PharmD
Aurélien Mary, PharmD, PhD
Giampiero Bricca, MD, PhD†
Saïd Kamel, PharmD, PhD
***Jeremy Bellien, PharmD, PhD**

*Service de Pharmacologie
Centre Hospitalier Universitaire de Rouen
1 rue de Germont
76031 Rouen Cedex
France
E-mail: jeremy.bellien@chu-rouen.fr

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and US Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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