The Oral Dipeptidyl Peptidase-4 Inhibitor Sitagliptin Increases Circulating Endothelial Progenitor Cells in Patients With Type 2 Diabetes

Possible role of stromal-derived factor-1α

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OBJECTIVE — Vasculoprotective endothelial progenitor cells (EPCs) are regulated by stromal-derived factor-1α (SDF-1α) and are reduced in type 2 diabetes. Because SDF-1α is a substrate of dipeptidyl-peptidase-4 (DPP-4), we investigated whether the DPP-4 inhibitor sitagliptin modulates EPC levels in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — This was a controlled, nonrandomized clinical trial comparing 4-week sitagliptin ($n = 16$) versus no additional treatment ($n = 16$) in addition to metformin and/or secretagogues in type 2 diabetic patients. We determined circulating EPC levels and plasma concentrations of SDF-1α, monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), and nitrites/nitrates.

RESULTS — There was no difference in clinical baseline data between the sitagliptin and control arms. After 4 weeks, as compared with control subjects, patients receiving sitagliptin showed a significant increase in EPCs and SDF-1α and a decrease in MCP-1.

CONCLUSIONS — Sitagliptin increases circulating EPCs in type 2 diabetic patients with concomitant upregulation of SDF-1α. This ancillary effect of DPP-4 inhibition might have potential favorable cardiovascular implications.

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Endothelial progenitor cells (EPCs) provide vascular protection by means of endothelial repair and neoangiogenesis (1). Type 2 diabetes, especially in the presence of macrovascular complications, is associated with reduced circulating EPCs (2), which in turn has been linked to incident cardiovascular disease (3,4). Reduced EPCs are considered a novel pathogenic mechanism of vascular disease and a biomarker of vascular risk (5). For these reasons, ways to stimulate EPCs in diabetes are actively pursued. The dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin blocks degradation of incretins by DPP-4. Among other physiological substrates of DPP-4 is stromal-derived factor-1α (SDF-1α) (6), a chemokine that stimulates bone marrow mobilization of EPCs (7). We have recently reported that reduction of circulating progenitor cells in diabetes is at least in part attributable to a bone marrow defect (8). Herein, we hypothesize that inhibition of DPP-4 mobilizes EPCs in patients with type 2 diabetes, by protecting SDF-1α from enzymatic degradation.

RESEARCH DESIGN AND METHODS — A detailed description of methods can be found in the online appendix available at http://care.diabetesjournals.org/cgi/content/full/dc10-0187/DC1. This was a controlled, nonrandomized, 4-week trial comparing 100 mg sitagliptin versus no additional treatment on top of metformin and/or secretagogues in poorly controlled type 2 diabetic patients. The protocol was approved by the Padova University Hospital ethics committee. At baseline and after 4 weeks, blood samples were drawn for determination of circulating EPCs and plasma concentrations of SDF-1α, vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), and nitrite/nitrate (NO_x). EPCs were defined as $CD34^+KDR^+$ cells and measured by flow cytometry as previously described (2). Total $CD34^+$ cell count was also determined, and $CD34^+$ or $CD34^+KDR^+$ cells were assayed for expression of CXCR4. SDF-1α, VEGF, and MCP-1 were measured using multiplex suspension arrays. NO_x was measured with an enzymatic assay. Plasma DPP-4 activity was measured as conversion of the substrate H-Gly-Pro-AMC to a fluorescent product. Data are expressed as means ± SE, and statistical significance was accepted at $P < 0.05$.

RESULTS — Clinical data of the sitagliptin and control groups are reported in online appendix Table 1, and there was no significant difference between the groups. The sample was representative of a 65-year-old diabetic population with mildly uncontrolled disease and a moderate prevalence of complications.

Therapy with sitagliptin 100 mg daily was well tolerated, and the patients reported no adverse effects. DPP-4 inhibi-

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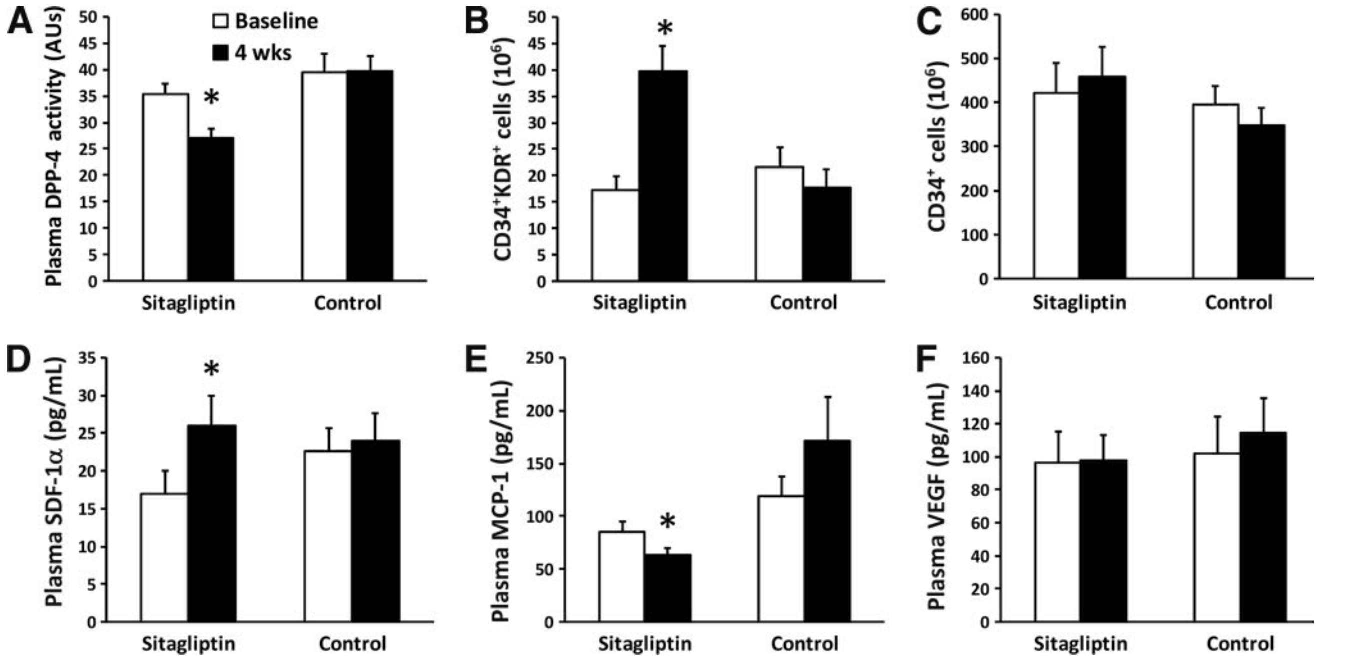

Figure 1—*Effects of sitagliptin on DPP-4 activity, progenitor cells, and soluble factors. Plasma free DPP-4 activity* (A)*, $CD34^+KDR^+$ EPCs levels* (B)*, $CD34^+$ cell levels* (C)*, and concentrations of SDF-1α* (D)*, MCP-1* (E)*, and VEGF* (F) *were determined at baseline and at 4 weeks in the sitagliptin intervention group and in the control group. *$P < 0.05$.*

tion was confirmed by a significant 23% reduction of free plasma DPP-4 activity in the sitagliptin group, while no change was found in the control group (Fig. 1*A*).

Progenitor cell counts were not different at baseline between the two groups. In the whole cohort, EPCs were significantly negatively correlated with baseline plasma glucose ($r = -0.445$; $P = 0.011$). Circulating EPCs increased about twofold in the sitagliptin group, and remained unchanged in the control group (Fig. 1*B*). The correlation between plasma glucose and EPCs was lost after 4 weeks (online appendix Fig. 1). Total $CD34^+$ cell count was unaffected in both groups (Fig. 1*C*). In the sitagliptin group, plasma concentrations of SDF-1α increased by 50% ($P <$ 0.001), while MCP-1 concentrations decreased by 25% ($P = 0.01$) and VEGF levels remained unchanged. No significant differences in baseline versus 4-week concentrations of SDF-1α, MCP-1, and VEGF were observed in the control group (Fig. 1*D–F*). We found no significant modification of NO_x concentrations in both groups. Online appendix Table 2 contains rough data showing that between-group differences of EPCs, SDF-1α, and MCP-1 were statistically significant. To explain the differential effects of sitagliptin on $CD34^+$ versus $CD34^+KDR^+$ cells, we show that SDF-1α receptor CXCR4 was expressed on 17% of $CD34^+$ cells and on 63% of $CD34^+KDR^+$ cells (online appendix Fig. 2).

CONCLUSIONS — In this study, we show for the first time that sitagliptin increases EPCs in type 2 diabetic patients, as an ancillary effect of DPP-4 inhibition, possibly mediated by the SDF-1α/CXCR4 axis.

Experimental studies demonstrate that EPCs stimulate endothelial repair and angiogenesis (1). These cells are reduced in diabetic patients at an early stage and are further impaired in patients with macro-/microvascular complications (2,8,9). Low baseline progenitor cell levels predict adverse outcomes of macro- and microangiopathy (3,4,10), and EPC reduction is now considered a novel route to development and worsening of diabetes complications. In response to ischemia, SDF-1α is upregulated and, upon binding to its receptor CXCR4, stimulates the bone marrow to release EPCs that are eventually recruited at ischemic sites (7). In diabetic animals a blunted SDF-1α response to ischemia is associated with inhibited progenitor cell release from the bone marrow and defective postischemic angiogenesis (11). Given that SDF-1α is a physiological substrate of DPP-4 (6), DPP-4 inhibition is expected to increase circulating SDF-1α levels, which in turn could affect EPC-mediate cardiovascular repair, as shown by Zaruba et al. (12) in mice with myocardial infarction.

We report that a 4-week therapy with 100 mg oral sitagliptin increased plasma SDF-1α concentration and circulating EPCs. The most straightforward interpretation is that DPP-4 inhibition raised SDF-1α concentrations, which mobilized EPC from the bone marrow. An alternative explanation is that glucose lowering per se improved EPC bioavailability (11,13). However, the short duration of the present trial and the loss of correlation between plasma glucose and EPC levels at study end seem to argue against this hypothesis. Finally, the increased glucagon-like peptide 1 concentrations achieved by DPP-4 inhibition might have determined an effect on EPCs through endothelial nitric oxide synthase activation (14), which is essential for EPC mobilization (15). So far, the absence of changes in nitrate/nitrite levels after sitagliptin does not support this hypothesis. Reduced concentrations of the proinflammatory chemokine MCP-1 achieved by sitagliptin is another potential mechanism for the restored levels of circulating EPCs. The mild and not significant action of sitagliptin on $CD34^+$ cells is to be attributed to the much lower expression of CXCR4 on these cells than on $CD34^+KDR^+$ EPCs. This result strengthens the hypothesis that sitagliptin modulates EPCs through the SDF-1α/CXCR4 axis.

This pilot trial was small and not randomized, and there was a relatively high drop-out rate. Thus, this ancillary effect of sitagliptin might have favorable cardiovascular implications but needs to be confirmed in larger and longer outcome studies.

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G.P.F. designed the study, recruited patients, collected and analyzed data, and wrote the manuscript. E.B. performed flow cytometry experiments and analyzed data. M.A. and L.M. performed soluble factor measurements and enzyme activity. V.F. and S.d.K. contributed to patients' recruitment, characterization, and data collection. C.A. standardized and supervised the flow cytometry protocols and interpreted data. A.T. supervised and reviewed the project. A.A. provided funds, interpreted results, and wrote the manuscript.

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