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Original Article

Dipeptidyl peptidase 4 concentration influenced by serum insulin levels rather than arterial stiffness index in type 2 diabetics

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Abstract: Objective: To investigate whether inhibitors of dipeptidyl peptidase-4 (DPP-4) can suppress the atherosclerotic development in diabetic patients. Methods: The prospective study was carried out in the Out-patient Department of XuHui Central Hospital, Shanghai, China, between March and August 2013. The correlation of major index of glucose and lipid metabolism profiles, and the arterial stiffness index (AI) between diabetic patients and healthy subjects were analyzed.Patients or Materials: 39 patients with type 2 diabetes and 29 healthy subjects were enrolled for measurements of blood glucose, plasma insulin, HbA1c, TC, cholesterol, HDL, AI and body mass index (BMI). Results: Significant differences were found between DPP-4 and blood glucose (fasting and 2 h postprandial), HbA1c, cholesterol, high density lipoprotein and arterial stiffness in normal subjects and diabetic patients. Only the fasting insulin concentration and high density lipoprotein had a significant impact on DPP-4 levels. Conclusions: It was clear that insulin (fasting) and HDL levels had an impact on DPP-4 activity but only in patients with Type 2 diabetes. The arterial stiffness index was not correlated with DPP-4 levels in Type 2 diabetic patients.

Keywords: Arterial stiffness index, cholesterol, DPP-4, HDL, insulin

Introduction

Atherosclerosis develops as a result of abnormal lipid metabolism and is exacerbated by microvascular diseases associated with diabetes [1]. Dipeptidyl peptidase-4 (DPP-4), expressed on the surface of most cell types, is an indiscriminate enzyme, which degrades a diverse range of substrates, such as glucagon-like peptide-1 (GLP-1). GLP-1 is responsible for increasing insulin secretion from the pancreas in a glucose-dependent manner [2]. Therefore, inhibitors of DPP-4 such as sitagliptin or vildagliptin will reduce the breakdown of GLP-1 and thus increase insulin levels indirectly [2]. However, it is of great interest whether DPP-4 inhibitors can influence lipid metabolism in patients with type 2 diabetes and also whether there is an association between DPP-4 and atherosclerosis. Although animal studies have revealed that inhibitors of DPP-4 can suppress the development of atherosclerotic plaques in diabetic ApoE knockout mouse models [3-5], this question has not been investigated in humans.

In the present study, we measured the preliminary index of blood lipid and glucose metabolism in patients with type 2 diabetes compared to normal subjects to analyze further the regression relationship between DPP-4 levels and the lipid metabolism profile.

Patients and methods

39 patients with type 2 diabetes (as the study group) and 29 healthy subjects (as the control group) were enrolled in the Out-patient Department of XuHui Central Hospital between March and August 2013. The inclusion criteria were that patients were 18 to 70 years old,

inactive (i.e. not participating in regular exercise), overweight to being mildly obese (BMI 27-35 kg/m^2), dyslipidemic (LDL cholesterol 130–-190 mg/dL or high density lipoprotein (HDL) cholesterol (40 mg/dL for men and 45 mg/dL for women), and postmenopausal status for women. Exclusion criteria included diagnosed diabetes or fasting glucose > 126 mg/dL, medications known to affect carbohydrate or lipid metabolism, on a diet, hypertension (blood pressure > 160/90 mmHg), known cardiovascular disease, tobacco use or musculoskeletal conditions that prohibited exercise training. The arterial stiffness index (AI) = [total cholesterol (TC) - HDL] L high density lipoprotein (HDL), AI is normally < 4, which indicates that the level of AI is not serious. The smaller the AI value, the lesser the degree of arterial stiffness, with the clinical risk of arterial stiffness causing heart and cerebral vascular diseases consequently being low. If the level of arterial stiffness is high, the risk of developing heart and cerebral vascular disease is increased.

Laboratory measurements

The patients' medical history, gender, age, weight and height were recorded. Routine measurements of blood glucose, plasma insulin, HbA1c, TC and HDL were performed using a fully automated biochemical analyser (Roche Modular P800, Roche Co. Shanghai, China).

DDP-4/CD26 assays method

After 8 hours of fasting, blood samples were collected from patients, centrifuged and the serum stored at -80°C for up to 8 weeks before analysis. The CD26 peptidase activity of cells was measured in 96 well microplates using the chromogenic substrate Gly-Pro-p-nitoanilide (Gly-Pro-pNA) (Enzo Life Sciences International, INC., PA, USA) according to the supplied manufacturer's protocol. Peptidase activity was expressed as pmol/min per 1,000 cells. Proteolytic activity was determined by measuring the amount of p-nitroanilide (pNA) formed in the supernatant at 405 nm. In the 96 well flat-bottomed plate, 10^4 cells/ml were incubated at 37°C with 4 mM Gly-Pro-pNA in 100 µL PBS buffer (pH 7.4) containing 10 mg/mL BSA. Absorbance was measured at 405 nm on a microplate spectrofluorometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA) every 2 min and pNA formed (in pmol) were calculated by comparison to a pNA standard curve. The results were plotted as pmol of pNA *vs*. min and the slope was calculated on the linear portion of the curve, giving a measure of DPPIV activity expressed as pmol/min per 1,000 cells. Tests were run on 3 triplicate samples (n = 3) for each sample and cell-free blanks and substrate-free blanks were run in parallel. Data are presented as the mean ± standard error of the mean (SEM) for all tests.

Artery IMT measurements

Color flow Doppler sonography (ACUSON Sequoia C512, Siemens Medical Solutions USA Inc., Mountain View, CA) was used for measurements using a high frequency probe, with the scan range set to include the common carotid artery, internal carotid artery, femoral artery and the dorsalis pedis artery. The distance from the boundary of the vascular intima and lumen to the boundary of tunica media and tunica adventitia is termed the IMT. IMT was measured 5 times (by the same doctor) to determine its average value.

Statistical analyses

Comparison of data within a group was performed using Student's *t*-test for unpaired data. Comparisons between healthy and diabetic patients were performed using Student's *t*-test for unpaired data. Simple (Pearson's) correlations coefficients were calculated using standard formulae. A multivariate linear regression analysis was carried out to evaluate independent associations between variables. All statistical analyses were performed using SPSS 13.0 software.

Results

Comparison of blood biochemistry indices and AI between normal subjects and diabetic patients

We found that there were significant differences between the DDP-4 fasting, DDP-4 (2 h postprandial), blood surge (fasting), blood surge (postprandial 2 h), peptide C (2 h postprandial), BMI, HbA1c, cholesterol, HDL and AI levels in the two groups (**Table 1**). The results indicate that diabetic patients have higher DDP-4 concentrations in the fasting group and 2 h postprandial and that increasing DDP-4 activity was associated with higher blood glucose levels

Table 1. Comparison of baselines between normal subjects and diabetic patients

	Control Group (*n* = 29)	Study Group (*n* = 39)	t or c^2	*P* value
Males [n, (%)]	4 (13.8)	22 (56.4)	12.7914	0.0003
Females [n, (%)]	25 (86.2)	17 (43.6)		
Age (years)	48 ± 13.38	56.26 ± 9.47	2.84	0.0067
DDP-4 (pmol/min GP-pNA)				
Fasting	530.84 ± 97.84	602.21 ± 155.21	2.32	0.0236
2 h postprandial	496.36 ± 130.46	625.67 ± 212.61	3.09	0.0029
Blood sugar (mmol/L)				
Fasting	4.89 ± 0.63	8.62 ± 2.9	7.51	< 0.0001
2 h postprandial	5.39 ± 1.01	15.55 ± 3.68	15.85	< 0.0001
Insulin (μI U/mL)				
Fasting	7.81 ± 4.06	12.06 ± 10.95	1.86	0.0717
2 h postprandial	37.71 ± 20.06	40.88 ± 28.05	0.48	0.633
Peptide C (ng/mL)				
Fasting	2.69 ± 0.81	2.82 ± 1.63	0.4	0.6923
2 h postprandial	9.59 ± 3.16	6.32 ± 3.19	3.99	0.0002
BMI (kg/m^2)	24.25 ± 2.88	26.54 ± 4.53	2.46	0.0166
HbA1c (%)	5.6 ± 0.36	8.85 ± 1.84	10.33	< 0.0001
Cholesterol (mmol/L)	1.77 ± 2.01	4.72 ± 1.01	7.21	< 0.0001
HDL (mmol/L)	1.47 ± 0.33	1.09 ± 0.3	4.8	< 0.0001
Arterial stiffness index (AI)	0.37 ± 1.77	5.04 ± 9.79	2.81	0.0079
Right artery IMT				
Common	0.767 ± 0.153	0.713 ± 0.214	0.42	0.6803
Internal	0.6 ± 0.1	0.578 ± 0.104	0.34	0.7362
Left carotid artery IMT				
Common	0.767 ±0.153	0.77 ± 0.291	0.02	0.9868
Internal	0.567 ± 0.058	0.6 ± 0.117	0.48	0.6353
Femoral artery IMT				
Right	0.675 ± 0.126	0.718 ± 0.094	0.82	0.4201
Left	0.675 ± 0.126	0.714 ± 0.097	0.73	0.4694
Dorsalis pedis artery IMT				
Right	0.25 ± 0.058	0.296 ± 0.092	0.97	0.3389
Left	0.25 ± 0.058	0.296 ± 0.092	0.97	0.3389

Note: the units of artery IMT measurements are in mm.

(**Table 1**). They also seems to be affected by their AI value but there was no obvious differences in the artery IMT measured in the two groups (**Table 1**). So we need to further analysis whether the change of DDP-4 has some relationship with the AI.

Effects of fasting insulin levels and HDL on DDP-4 concentrations in diabetic patients

In order to understand the effects of blood glucose (during fasting and 2 h postprandial) and the lipid metabolism index in normal subjects and patients with diabetes on DDP-4 activity, we carried out a regression analysis. We found that during fasting, the DPP-4 concentrations in patients with diabetes were not affected as determined by the factors measured. The concentration of DDP-4 was correlated with the left and the right common carotid artery IMT in the control group (**Table 2**). Postprandially, the blood concentration of DDP-4 was affected by fasting insulin and HDL levels only in the study group (**Table 3**).

AI did not influence DDP-4 concentration

Although basic AI was significantly higher in the study group compared to the control group (**Table 1**), AI did not affect DPP4 activity in either group (**Tables 2** and **3**).

Table 2. Effect of variables on DDP-4 (fasting) between normal subjects and diabetic patients

	Normal Subjects		Diabetic Patients	
	Standardized regression coefficients	*P* values	Standardized regression coefficients	*P* values
Age (in years)	0.022	0.912	0.072	0.665
Blood sugar (mmol/L)				
Fasting	-0.205	0.287	0.142	0.409
2 h postprandial	0.093	0.632	-0.112	0.516
Insulin (μl U/mL)				
Fasting	-0.097	0.623	0.265	0.19
2 h postprandial	-0.306	0.114	-0.199	0.329
Peptide C (ng/mL)				
Fasting	0.035	0.861	0.234	0.191
2 h postprandial	-0.247	0.214	-0.091	0.61
BMI (kg/m^2)	0.106	0.59	0.09	0.602
HbA1c (%)	0.252	0.187	0.3	0.076
Cholesterol (mmol/L)	0.085	0.662	-0.017	0.923
HDL (mmol/L)	0.054	0.779	0.068	0.695
Arterial stiffness index (AI)	0.128	0.509	-0.223	0.192
Right artery IMT				
Common	-1	0.011	0.185	0.398
Internal	-0.979	0.132	0.162	0.462
Left carotid artery IMT				
Common	-1	0.011	0.15	0.495
Internal	-0.951	0.201	0.129	0.557
Femoral artery IMT				
Right	0.057	0.943	0.077	0.698
Left	0.057	0.943	0.066	0.739
Dorsalis pedis artery IMT				
Right	0.6	0.4	-0.195	0.319
Left	0.6	0.4	-0.195	0.319

Note: the units of artery IMT measurements are in mm.

Discussion

Although DPP-4 inhibitors have been demonstrated to improve atherosclerosis lesions in mice diabetes models, the effects of these agents on human diabetic patients with atherosclerosis have not been well documented. In the present study, we investigated the primary relationship between lipid metabolism and DPP-4 levels in patients with type 2 diabetes compared to healthy subjects. Unlike the results reported in mouse studies, it was not possible to establish a significant correlation between measured lipid profiles and DPP-4 levels after fasting, even though the AI index was significantly higher in the diabetic patient group than in the control group. However, at 2 h postprandial, insulin (fasting) and HDL were shown to be significant impact factors on DPP-4 activity in patients with diabetes. In other words, the effect of insulin (fasting) and HDL on DPP-4 level was increased 2 h postprandial.

Table 1 shows that there are significant differences between DPP-4 (fasting, 2 h postprandial), blood glucose (fasting, 2 h postprandial), insulin (fasting, 2 h postprandial), peptide C (fasting, 2 h postprandial), BMI, HbA1c, cholesterol, HDL and AI in the control and study group. However, it is noteworthy that DPP-4 levels did not change after fasting or 2 h postprandial in either group. We would like to point out that AI was much higher in the study group compared to the control group (**Table 1**) but there was no detectable effect of AI volume on DPP-4 activity (**Tables 2** and **3**), indicating no connection between these two factors. Likewise, there was no significant effect of any lipid profile index on DPP-4 levels in the present study. The relatively small sample size perhaps is the main reason for this finding. However, Barbieri et al. [6] examined the effects of DPP-4 inhibitors on IMT in 90 patients with type 2 diabetes. Their results showed that after 3 months of treatment with sitagliptin or vildagliptin, the levels of several interleukin factors were significantly changed, but the lipid profiles did not show any improvement. Moreover, multivariate analysis demonstrated that all the measured factors, with the exception of delta MAGE, were not correlated with IMT. Similarly, Katakami et al.

Table 3. Effect of variables on DDP-4 (postprandial 2 h) between normal subjects and diabetic patients

	Normal Subjects		Diabetic Patients	
	Standardized regression coefficients	*P* values	Standardized regression coefficients	*P* values
Age (in years)	0.297	0.118	0.023	0.888
Blood sugar (mmol/L)				
Fasting	-0.249	0.193	-0.278	0.101
2 h postprandial	0.287	0.132	-0.055	0.749
Insulin (μI U/mL)				
Fasting	-0.212	0.279	0.441	0.024
2 h postprandial	-0.038	0.846	-0.091	0.659
Peptide C (ng/mL)				
Fasting	-0.026	0.897	0.184	0.304
2 h postprandial	0.036	0.86	-0.06	0.738
BMI (kg/m^2)	0.272	0.161	0.18	0.293
HbA1c (%)	0.168	0.384	0.081	0.639
Cholesterol (mmol/L)	-0.248	0.913	0.405	0.46
HDL (mmol/L)	-0.021	0.566	-0.127	0.035
Arterial stiffness index (AI)	0.111	0.924	0.353	0.142
Right artery IMT				
Common	-0.019	0.757	-0.25	0.84
Internal	-0.372	0.636	-0.045	0.906
Left carotid artery IMT				
Common	-0.541	0.757	0.026	0.587
Internal	-0.372	0.97	0.12	0.481
Femoral artery IMT				
Right	-0.048	0.474	0.155	0.903
Left	0.526	0.474	-0.024	0.825
Dorsalis pedis artery IMT				
Right	0.526	0.694	-0.044	0.585
Left	-0.306	0.694	-0.108	0.585

Note: the units of artery IMT measurements are in mm.

investigated the effects of alogliptin on diabetic atherosclerosis in 172 patients with type 2 diabetes, but their eagerly awaited results on lipid metabolisms are still not available [7].

The pharmacological mechanism of action of DPP-4 inhibitors is to increase incretin levels (GLP-1 and GIP) [8, 9], which inhibits glucagon release, decreases gastric emptying and reduces blood glucose levels and DPP-4 inhibitors have been approved by the FDA [10]. Although several studies have reported that DPP-4 inhibitors decrease atherosclerosis lesions in mouse diabetes models, the mechanism(s) underlying this action remain un explained. Moreover, there is no clear evidence that DPP-4 inhibitors attenuate the development of atherosclerosis in type 2 diabetic patients. The possible reasons are: first, there are huge differences between human beings and mice; second, there may be fundamental differences between human diabetes and diabetic mouse models.

Conclusions

The main finding is that there is a difference between two groups on the basis of blood lipid and the glucose metabolism indices. It is clear that insulin (fasting) and HDL have an impact on DPP-4 activity but only in patients with type 2 diabetes, findings that are consistent with previous reports. One limitation of the present study is the small sample size employed.

Discloaure of conflict of interest

None.

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References

[1] Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC and Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000; 321: 405-412.

[2] Toft-Nielsen MB, Madsbad S and Holst JJ. Determinants of the effectiveness of glucagon-

like peptide-1 in type 2 diabetes. J Clin Endocrinol Metab 2001; 86: 3853-3860.
[3] Terasaki M, Nagashima M, Nohtomi K, Kohashi K, Tomoyasu M, Sinmura K, Nogi Y, Katayama Y, Sato K, Itoh F, Watanabe T and Hirano T. Preventive effect of dipeptidyl peptidase-4 inhibitor on atherosclerosis is mainly attributable to incretin's actions in nondiabetic and diabetic apolipoprotein E-null mice. PLoS One 2013; 8: e70933.
[4] Ta NN, Li Y, Schuyler CA, Lopes-Virella MF and Huang Y. DPP-4 (CD26) inhibitor alogliptin inhibits TLR4-mediated ERK activation and ERK-dependent MMP-1 expression by U937 histiocytes. Atherosclerosis 2010; 213: 429-435.
[5] Zeng Y, Li C, Guan M, Zheng Z, Li J, Xu W, Wang L, He F and Xue Y. The DPP-4 inhibitor sitagliptin attenuates the progress of atherosclerosis in apolipoprotein-E-knockout mice via AMPK- and MAPK-dependent mechanisms. Cardiovasc Diabetol 2014; 13: 32.
[6] Barbieri M, Rizzo MR, Marfella R, Boccardi V, Esposito A, Pansini A and Paolisso G. Decreased carotid atherosclerotic process by control of daily acute glucose fluctuations in diabetic patients treated by DPP-IV inhibitors. Atherosclerosis 2013; 227: 349-354.
[7] Katakami N, Mita T, Yoshii H, Onuma T, Kaneto H, Osonoi T, Shiraiwa T, Kosugi K, Umayahara Y, Yamamoto T, Yokoyama H, Kuribayashi N, Jinnouchi H, Gosho M, Watada H, Shimomura I; Collaborators on the Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis Trial. Rationale, design, and baseline characteristics of a trial for the prevention of diabetic atherosclerosis using a DPP-4 inhibitor: the Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis (SPEAD-A). J Atheroscler Thromb 2013; 20: 893-902.
[8] Behme MT, Dupre J and McDonald TJ. Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. BMC Endocr Disord 2003; 3: 3.
[9] Dupre J, Behme MT, Hramiak IM, McFarlane P, Williamson MP, Zabel P and McDonald TJ. Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. Diabetes 1995; 44: 626-630.
[10] Dicker D. DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. Diabetes Care 2011; 34 Suppl 2: S276-278.