ARTICOLI ORIGINALI

Nephroprotection with saxagliptin

Nicolangelo Iazzetta[1], Carlo Garofalo[2], Manuela Savino[2], Adelia Sagliocca[2], Sara Santangelo[2], Mario Pacilio[2], Maria Elena Liberti[2], Andrea Camocardi[2], Camilla Ambrosca[1], Roberto Minutolo[2], Luca De Nicola[2], Giuseppe Conte[2]

(1) Servizio di Medicina Interna; P.O. S.M.d.P. Incurabili e P.O. Loreto Crispi, Napoli
(2) Cattedra di Nefrologia - Seconda Università di Napoli

Corrispondenza a: Prof. Giuseppe Conte; Cattedra di Nefrologia, Seconda Università di Napoli Dip. Scienze Mediche, Chirurgiche, Metaboliche e dell'invecchiamento - Piazza Miraglia 80138 Napoli; Tel:+39 0812549409 Fax:+39 0812549409 Mail: giuseppe.conte@unina2.it

Abstract

The nephroprotective effect of the new anti-diabetic drugs acting on incretin system is suggested by pre-clinical studies. However, no study evaluating kidney effects of these drugs as primary outcome on the long term has been conducted in patients followed in diabetes centers. We designed a pilot observational study involving two diabetes clinics to evaluate the effect of prolonged treatment with saxagliptin on renal function in type 2 diabetics. Patients were enrolled if treated for at least 12 months with saxagliptin without concurrent changes to anti-hypertensive and lipid-lowering therapy. Primary outcome was to evaluate the effect of saxagliptin on albuminuria and estimated glomerular filtration rate (eGFR). Secondary outcomes were the effects of treatment on common clinical and laboratory parameters. Sixty-three patients were enrolled. After 12 months of treatment with saxagliptin, albuminuria declined from a mean (95%CI) of 39 (25-52) to 22 (14-30) mg/l (P<0.001), and the prevalence of increased albuminuria (>20 mg/L) diminished by 27% versus baseline. The anti-albuminuric effect was independent of glycemic and blood pressure control. The eGFR remained unchanged after treatment in the presence of decreased glycated hemoglobin (from 7.1 to 6.7%). Therefore, this pilot study suggests that saxagliptin treatment in diabetic patients at high renal risk is associated with a reduction in albuminuria and GFR stability. Prospective trials are required to confirm the potential nephroprotective effects of saxagliptin.

Key words: diabetes mellitus, DPP-IV inhibitors, kidney function, saxagliptin

Introduction

During the last years, various classes of anti-diabetic drugs (AD) have been marketed in order to optimize glycemic control in patients with diabetes mellitus (DM) type 2. Nevertheless, despite an improvement of the glycemic profile, almost one-third of diabetic patients still develops micro-vascular complications such as diabetic nephropathy [1], that is the leading cause of end stage renal disease (ESRD) [2]. Until now, there are no available clinical evidences on the long-term nephroprotective effects of the new AD. In particular, experimental studies have shown both in vitro and in rats the anti-fibrotic and anti-proteinuric effects of inhibitors of the enzyme dipeptidyl peptidase 4 (DPP4i) [3] (full text) [4] (full text) [5] [6] (full text). However, despite some papers with short follow-up have shown a reduction of the albuminuria in the subjects treated with DPP4i [7] (full text) [8] [9] [10], no study has evaluated the renal effects of long term treatment with DPP4i as primary

endpoint. A proper evaluation of the nephroprotective effect of DPP4i needs the absence of variations of other anti-albuminuric therapies, such as anti-hypertensive drugs and statins.

We designed a pilot study to verify the effect of one year treatment with saxagliptin, a DPP4 inhibitor, on renal function parameters in a cohort of patients affected from DM type 2 and followed in two diabetes centers.

Material and methods

Inclusion and exclusion criteria

We have conducted an observational retrospective study including patients followed in diabetology clinics at "S.M.d.P. Incurabili" and "Loreto Crispi" hospital in Naples. Recruited patients were affected by diabetes mellitus type 2 and treated for at least 1 year with traditional AD, with the addition in therapy of saxagliptin and that had been treated, without changes and over the last 6 months with anti-hypertensive and lipid-lowering agents. Patients were excluded if affected by DM type 1, gestational diabetes, neoplasia, advanced cirrhosis, congestive heart failure. Hypertension was defined by blood pressure >140/90 mmHg or treatment with anti-hypertensive; hyperlipidemia has been defined by the necessity of lipid-lowering therapy.

Primary outcome

The primary outcome of the study was to evaluate the change of albuminuria and estimated glomerular filtration rate (eGFR). GFR has been estimated by CKD-EPI formula according to the methodology of Skali and coll. [11]. The value of albuminuria, (milligrams/liter) has been used to classify the patients, as indicated by K-DIGO guidelines 2012 [12], in three categories: normoalbuminuria (A1) <20 mg/L, albuminuria moderately increased (A2) >20 and <200 mg/L, albuminuria severely increased (A3) >200 mg/L.

Statistic analyses

Continuous variables with normal distribution have been reported as mean and standard deviation except for albuminuria reported as mean and 95% confidence interval (CI) and analyzed with T test for dependent sample. Those with non Gaussian distribution have been reported as median and interquartile range and analyzed with Wilcoxon test; categorical variables have been reported as percentages and compared with McNemar test. A linear multivariate regression test has been used to verify the determinants of absolute change in albuminuria in comparison to the baseline level; model was done *a priori* including the variation of the glycated hemoglobin (HbA1c), variations in the systolic blood pressure (BP), age, sex, number of anti-hypertensive drugs, therapy with RAS-inhibitors at baseline and category of albuminuria. P value <0.05 has been considered significant.

Results

Sixty-three patients with DM type 2, diagnosed in median from 6 years [IQR, 4-14], were enrolled. The mean age was 65.4±9.9 years. 71% patients were hypertensive treated with anti-hypertensive drugs while 65% were treated with statins. None of the included subjects had a previous history of major cardiovascular events. Five patients (7.9%) had basal eGFR <60 ml/min/1.73m^2. As shown in Table 1, after one year of treatment with saxagliptin, we observed a significant reduction of HbA1c, albuminuria, systolic BP and LDL values. eGFR remained substantially unchanged. At baseline, majority of patients (56%) were in class A2 (Figure 1).

After one year of treatment, prevalence of patients with albuminuria A1 increased while prevalence of class A2 albuminuria decreased significantly (Figure 1).

Albuminuria reduction was associated only with basal class of albuminuria while being independent from the glycemic and BP control and from the anti-hypertensive therapy (Table 2). Treatment followed by enrolled subjects is depicted in Table 3.

Discussion

Our study suggests a potential nephroprotective effect of saxagliptin. After one year of treatment with this agent, the significant antialbuminuric effect was observed in absolute terms and as prevalence of patients with class A2 albuminuria (20-200 mg/L). The effect was independent from BP changes, use of RAS-inhibitors as well as from the improvement of glycemic control.

One year of follow up is not sufficient to examine the effect of a drug on the progression of chronic kidney disease; nevertheless, it is interesting that eGFR remained unchanged. This finding becomes remarkable when considering that enrolled subjects had a high risk of progression, being diabetics with a high percentage of albuminuria (59% of the cohort at

Tabella 1. Main parameters at baseline and after 1 year of treatment with saxagliptin

	Basal	Final	P
Weight (kg)	81.2±17.2	80.6±17.2	0.088
Hb1Ac (%)	7.1±1.0	6.7±0.7	<0.001
SBP (mmHg)	143±16	137±13	0.004
DBP (mmHg)	81±9	79±6	0.085
LDL (mg/dl)	91±30	85±28	0.020
TG (mg/dl)	125 [98-174]	131 [94-189]	0.336
eGFR (ml/min)	84±16	85±16	0.123
Albuminuria (mg/L)	38 (25-52)	22 (14-30)	<0.001

A TG: triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure. GFR was estimated by CKD-EPI. Weight, Hb1Ac, SBP, DBP, LDL, eGFR values are mean and standard deviation; TG is median and interquartile range; albuminuria is mean and 95% confidence interval.

Tabella 2. Multivariate linear regression analysis of variations in albuminuria determinants, after one-year treatment with saxagliptin

	R	P
Age	0.077	0.478
Gender	0.022	0.828
Change in SBP	-0.047	0.651
Number of anti-HT drugs	-0.007	0.961
Use of anti-RAS	-0.060	0.686
Change in HbA1C	-0.049	0.642
Albuminuria class at baseline	-0.676	<0.001
Costant		0.664

SBP: systolic blood pressure; HbA1c: glycated emoglobin, HT: hypertension; RAS: renin angiotensin system.

baseline). Given the retrospective nature of the study, it is only possible to hypothesize an association between the saxagliptin and improvement of renal damage, while a causal relationship cannot be established. Randomized clinical trials are definitely needed to verify the nephroprotective effect.

As suggested by some experimental evidences, use of DPP4i induces effects that go beyond the better glycemic control; in particular, their effect on development and progression of micro-albuminuria, that is recognized to be as main marker of renal damage and increased cardiovascular risk [13], suggests a potential nephroprotective role of this class of drugs.

Tabella 3. Anti-hypertensive, anti-diabetic and lipid-lowering drugs

Drug	% of patient
Metformin	100
Sulfonylureas	10
Glitazones	2
Ace-inhibitors	18
ARBs	46
Diuretics	22
Beta-blockers	22
CCB	19
Other anti-HT drugs	19
Statins	65

ARBs, angiotensin 2 receptor blockers; CCB: calcium channel blockers; HT: hypertension.

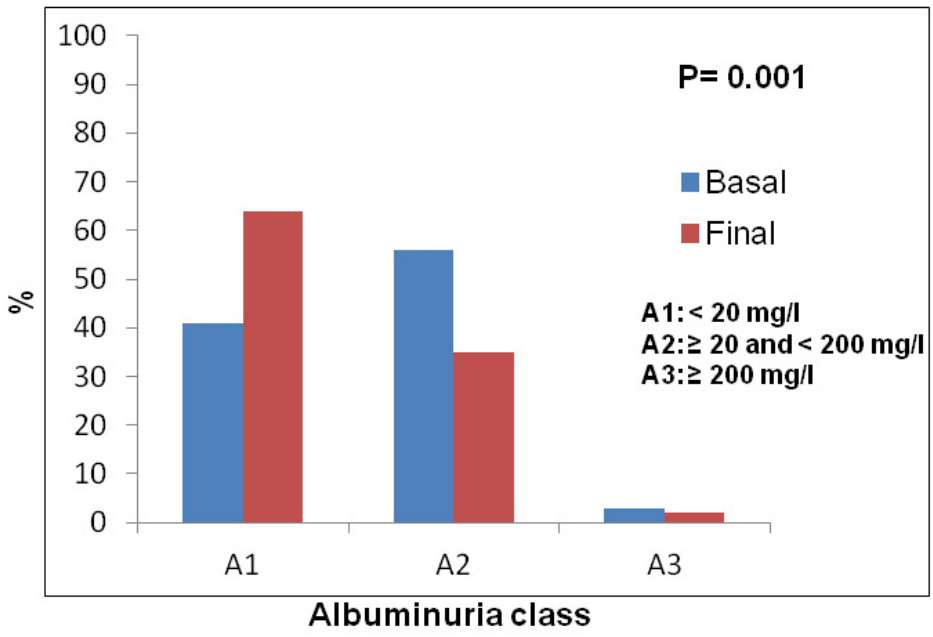

Figura 1.
Variations in the percentage of albuminuria classes after 1 year of treatment with saxagliptin.

The main mechanism of the DPP4i is the enhancement of the action of incretins, mainly native GLP-1, by lowering their degradation. In the kidneys, GLP-1 receptors are expressed on glomerular endothelial cells, mesangial cells and also on podocytes and proximal tubular cells. Of note, a lower expression of GLP-1 receptors has been demonstrated in the diabetic rat [14] (full text). More important, different studies have shown GLP-1 involvement in determining a reduced production of advanced glycation end products (AGEs) through the activation of the Protein Kinases A (PKA) and its anti-inflammatory properties with the stimulation of nitric oxide production and inhibition of production of angiotensin 2, PAI-1, ICAM-1 and VCAM-1 [15] [16]. Hence, DPP4i, through an increase of circulating levels of GLP-1, may have a role in reducing inflammatory pathways (increased production of free radicals of the oxygen, excessive production of AGEs) that represents the first step of the endothelial damage and the consequent evolution toward fibrosis. It is important to underline that the renal effects of the DPP4i are only in part related to their action on the circulating levels of GLP-1. In fact, it has been shown an incretin-independent effect of the enzyme dipeptidyl peptidase 4 on hormones, neuropeptidases, citokines and chemokines [17] [18]. DPP4 is bound on the surface of different cellular types, in particular proximal tubular and endothelial cells [19] (full text). Hence, an up-regulation of the glomerular expression of DPP4 has been shown in inflammatory states [18]. Finally, a greater urinary concentration of DPP4 has been found in diabetic patients versus non-diabetics and it is positively correlated with the degree of albuminuria [19] (full text). Recently, Shi and coll. have underlined [20] that in diabetic rats the inhibition of the enzyme DPP4 is associated to a reduction of the level of renal fibrosis dependent on the interaction between enzyme and integrin beta1 that stimulates the release of TGF-beta, a fibrotic factor. In an additional study in diabetic rats, linagliptin reduced albuminuria when combined with angiotensin II receptor blockers (ARBs); histologically,inhibition of DPP4 was associated to a reduced glomerulosclerosis and a reduction of the levels of TNF-alpha suggesting an anti-inflammatory and antioxidant effect [5]. Furthermore, some observational studies in patients have highlighted a potential beneficial effect of DPP4i. In particular, a first study, including 36 patients with HbA1c >6,5% under 50 mg/day of sitagliptin for six months [7] (full text), showed a significant reduction of HbA1c, BP and urine protein-to-creatinine ratio. A second prospective study in 82 subjects, treated for 52 weeks with saxagliptin as add-on therapy, had as primary endpoint the variations of the HbA1c and as secondary endpoint the changes of BMI, BP and ACR [8]. A reduction of all the three parameters was observed. Groop et al. performed a systematic analysis of double blind randomized studies with a duration of 24-52 weeks on the effects of linagliptin used in monotherapy or added to other hypoglycemic oral drugs [9]; the study included diabetic patients with persistent albuminuria (30-3000 mg/g) in treatment with an ACE-inhibitor or ARB; 168 patients assumed linagliptin while 59 placebo. Primary endpoint was the variation in the percentage of the geometric mean of ACR after 24 weeks of treatment. Analyses show that linagliptin allowed ACR to decrease of 32%. The importance of this reduction did not correlate with HbA1C change. Of note, the large SAVOR-TIMI 53 trial has evidenced a reduction in development and progression of the micro-albuminuria in the group treated with saxagliptin in comparison to the placebo [21]. Finally, a recent meta-analysis of thirteen trials (5466 patients) was aimed at evaluating the effect of linagliptin on the combined renal endpoint of development of albuminuria A2, albuminuria A3, increase of creatinine to 250 mcmol/L, reduction of the 50% of the GFR, incidence of acute renal failure and death [22] (full text). Authors have calculated a hazard ratio (HR) for the combined endpoint of 0.84 (95%CI 0.72-0.97) for the linagliptin in comparison to the group treated with placebo; moreover, in subjects treated with linagliptin the risk was reduced when the appearance or moderate increase of albuminuria was considered as single endpoint, with HR 0.82 (CI, 0.69-0.98). A new trial (MARLINA) is currently in progress; the aim

is to define the renal effects of DPP4 inhibition [23]; it is expected an enrollment of 350 patients, with no controlled DM and evidence of renal disease, randomized to linagliptin 5 mg or placebo; the change of HbA1c and ACR after 24 weeks of treatment will be examined.

Our study, for the first time, has evaluated as primary endpoint the two main indicators of renal prognosis (albuminuria and eGFR) in a cohort of diabetic subjects treated with saxagliptin for one year. Strength is that patients were enrolled if anti-hypertensive and lipid-lowering therapy remained unmodified throughout the entire duration of the follow-up; we have therefore limited the possible influence of therapeutic variations on the results obtained. Several limitations must be, however, considered. Indeed, the retrospective nature of the study prevents to draw any causal relationship between administration of saxagliptin and improvement of renal parameters; furthermore, the absence of a control group and the relatively small sample do not allow to exclude the influence of confounding factors; finally, the measurement of the parameters of renal function was obtained in a single visit.

Conclusions

This pilot study suggests that one-year therapy with saxagliptin reduces albuminuria in type 2 diabetic patients. Randomized clinical trials are necessary to verify the nephroprotective potential of saxagliptin as other inhibitors of DPP4.

Bibliografia

[1] De Cosmo S, Rossi MC, Pellegrini F et al. Kidney dysfunction and related cardiovascular risk factors among patients with type 2 diabetes. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2014 Mar;29(3):657-62

[2] US Renal Data System. USRDS 2013 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. Bethesda, MD: US Renal Data System, 2013

[3] Mega C, de Lemos ET, Vala H et al. Diabetic nephropathy amelioration by a low-dose sitagliptin in an animal model of type 2 diabetes (Zucker diabetic fatty rat). Experimental diabetes research 2011;2011:162092 (full text)

[4] Liu WJ, Xie SH, Liu YN et al. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in streptozotocin-induced diabetic rats. The Journal of pharmacology and experimental therapeutics 2012 Feb;340(2):248-55 (full text)

[5] Alter ML, Ott IM, von Websky K et al. DPP-4 inhibition on top of angiotensin receptor blockade offers a new therapeutic approach for diabetic nephropathy. Kidney & blood pressure research 2012;36(1):119-30

[6] Glorie LL, Verhulst A, Matheeussen V et al. DPP4 inhibition improves functional outcome after renal ischemia-reperfusion injury. American journal of physiology. Renal physiology 2012 Sep;303(5):F681-8 (full text)

[7] Hattori S Sitagliptin reduces albuminuria in patients with type 2 diabetes. Endocrine journal 2011;58(1):69-73 (full text)

[8] Harashima SI, Ogura M, Tanaka D et al. Sitagliptin add-on to low dosage sulphonylureas: efficacy and safety of combination therapy on glycaemic control and insulin secretion capacity in type 2 diabetes. International journal of clinical practice 2012 May;66(5):465-76

[9] Groop PH, Cooper ME, Perkovic V et al. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. Diabetes care 2013 Nov;36(11):3460-8

[10] Deacon CF Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes, obesity & metabolism 2011 Jan;13(1):7-18

[11] Skali H, Uno H, Levey AS et al. Prognostic assessment of estimated glomerular filtration rate by the new Chronic Kidney Disease Epidemiology Collaboration equation in comparison with the Modification of Diet in Renal Disease Study equation. American heart journal 2011 Sep;162(3):548-54

[12] Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members et al. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Annals of internal medicine 2013 Jun 4;158(11):825-30

[13] Drury PL, Ting R, Zannino D et al. Estimated glomerular filtration rate and albuminuria are independent predictors of cardiovascular events and death in type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetologia 2011 Jan;54(1):32-43

[14] Mima A, Hiraoka-Yamomoto J, Li Q et al. Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCβ activation in diabetes. Diabetes 2012 Nov;61(11):2967-79 (full text)

[15] Erdogdu O, Nathanson D, Sjöholm A et al. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. Molecular and cellular endocrinology 2010 Aug 30;325(1-2):26-35

[16] Kodera R, Shikata K, Kataoka HU et al. Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. Diabetologia 2011 Apr;54(4):965-78

[17] Tagore DM, Nolte WM, Neveu JM et al. Peptidase substrates via global peptide profiling. Nature chemical biology 2009 Jan;5(1):23-5

[18] Hocher B, Reichetzeder C, Alter ML et al. Renal and cardiac effects of DPP4 inhibitors--from preclinical development to clinical research. Kidney & blood pressure research 2012;36(1):65-84

[19] Sun AL, Deng JT, Guan GJ et al. Dipeptidyl peptidase-IV is a potential molecular biomarker in diabetic kidney disease. Diabetes & vascular disease research 2012 Oct;9(4):301-8 (full text)

[20] Shi S, Srivastava SP, Kanasaki M et al. Interactions of DPP-4 and integrin β1 influences endothelial-to-mesenchymal transition. Kidney international 2015 Sep;88(3):479-89

[21] Scirica BM, Bhatt DL, Braunwald E et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. The New England journal of medicine 2013 Oct 3;369(14):1317-26 (full text)

[22] Cooper ME, Perkovic V, McGill JB et al. Kidney Disease End Points in a Pooled Analysis of Individual Patient-Level Data From a Large Clinical Trials Program of the Dipeptidyl Peptidase 4 Inhibitor Linagliptin in Type 2 Diabetes. American journal of kidney diseases : the official journal of the National Kidney Foundation 2015 Sep;66(3):441-9 (full text)

[23] clinicaltrials.gov