Faculdade de Medicina de São José do Rio Preto
Programa de Pós-graduação em Ciências da Saúde

LUCIANA NEVES COSENSO MARTIN

EFEITOS DA VILDAGLIPTINA NA FUNÇÃO ENDOTELIAL, RIGIDEZ ARTERIAL E NA PRESSÃO ARTERIAL EM PACIENTES COM DIABETES MELLITUS DO TIPO 2 E HIPERTENSÃO ARTERIAL

São José do Rio Preto
2017

LUCIANA NEVES COSENSO MARTIN

EFEITOS DA VILDAGLIPTINA NA FUNÇÃO ENDOTELIAL, RIGIDEZ ARTERIAL E NA PRESSÃO ARTERIAL EM PACIENTES COM DIABETES MELLITUS DO TIPO 2 E HIPERTENSÃO

Tese apresentada à Faculdade de Medicina de São José do Rio Preto para obtenção do título de Doutor no Curso de Pós-Graduação em Ciências da Saúde, Eixo Temático: Medicina Interna.

Orientador: Prof. Dr. José Fernando Vilela Martin

São José do Rio Preto
2017

Martin, Luciana Neves Cosenso.
Efeitos da vildagliptina na função endotelial, rigidez arterial e na pressão arterial em pacientes com diabetes mellitus do tipo 2 e hipertensão arterial./ Luciana Neves Cosenso Martins.
São José do Rio Preto, 2017.
53 p.

Tese (Doutorado) – Faculdade de Medicina de São José do Rio Preto – FAMERP
Eixo Temático: Medicina Interna
Orientador: Prof. Dr. José Fernando Vilela Martin

1. Inibidor de DPP-4; 2. Função endotelial; 3. Vildagliptina; 4. Diabetes mellitus do tipo 2; 5. Hipertensão arterial; 6. Rigidez arterial

LUCIANA NEVES COSENSO MARTIN

EFEITOS DA VILDAGLIPTINA NA FUNÇÃO ENDOTELIAL, RIGIDEZ ARTERIAL E NA PRESSÃO ARTERIAL EM PACIENTES COM DIABETES MELLITUS DO TIPO 2 E HIPERTENSÃO

BANCA EXAMINADORA

TESE PARA OBTENÇÃO DO GRAU DE DOUTOR

Presidente e Orientador: Prof. Dr. José Fernando Vilela Martin

2º Examinador: Prof. Dr. Heitor Moreno Júnior

3º Examinador: Prof. Dr. Antônio Carlos Palandri Chagas

4º Examinador: Prof. Dr. Antônio Carlos Pires

5º Examinador: Prof. Dr. Juan Carlos Yugar Toledo

Suplentes: Prof. Dr. José Paulo Cipullo e Prof. Dr. Rui Manoel dos Santos Póvoa

São José do Rio Preto, 02/02/2017.

SUMÁRIO

- ✓ **Ao meu esposo, orientador, pela dedicação, paciência e companheirismo.**

- ✓ **Aos meus filhos, Fernando Lucas e Letícia, que meu esforço seja exemplo para suas vidas.**

Agradecimentos

- ✓ A Deus, criador de tudo.

- ✓ Ao meu orientador, Prof. Dr. José Fernando Vilela Martin pelo exemplo.

- ✓ Ao amigo Tadeu, por realizar e tabular os exames de função endotelial.

- ✓ Aos funcionários do CIP Funfarme/Famerp, pelo auxílio no atendimento aos pacientes do estudo, especialmente às enfermeiras Débora e Cristiane.

- ✓ Ao colega Marcelo, pela consultoria em estatística, orientando e estimulando na conclusão deste estudo.

- ✓ À Faculdade de Medicina de São José do Rio Preto – Famerp, possibilidade de realização deste trabalho.

- ✓ A todos os professores da pós-graduação da Famerp, pelas aulas e bons exemplos, em especial a Prof. Dra. Dorotea Rossi Silva Souza pelo incentivo.

- ✓ A professora Lilian e ao colega Maurício, pela realização da estastística.

- ✓ Aos pacientes participantes desse estudo.

- ✓ À professora Cláudia Cesarino, enfermeira Cleo, colega Danielli e aos residentes da endocrinologia, pelo auxílio na captação de pacientes para a pesquisa.

- ✓ Aos colegas da clínica médica, pela oportunidade e confiança em meu trabalho.

- ✓ Aos colegas da endocrinologia, pelo conhecimento e amizade compartilhados.

- ✓ Aos funcionários da pós-graduação Fabiana, José Antônio e Luís Henrique, pelo empenho na realização de seu trabalho.

- ✓ Aos meus pais, José Roque e Azilá, pelo apoio e ajuda incondicional.

- ✓ Aos meus queridos filhos, Fernando Lucas e Letícia, por todo seu amor.

- ✓ Ao laboratório Novartis, pelo apoio financeiro.

Epígrafe

"...quando você eliminou o impossível, tudo o que restar, por mais improvável, deve ser a verdade."

Umberto Eco

Artigo 1

Artigo 2

Artigo 3

Artigo 2

Artigo 3

AIx - Augmentation index

AIx@75 - Augmentation index corrigido pela frequência cardíaca de 75 bpm

AS - Arterial stiffness

cSBP - Central systolic blood pressure

DAC - Doença arterial coronariana

DCV - Doença Cardiovascular

DM 2 - Diabetes mellitus do tipo 2

DPP-4 - Dipeptidyl peptidase-4

FMD - Flow-mediated vasodilation

GIP - Glucose-dependent insulinotropic polypeptide

GLP-1 - Glucagon-like peptide-1 (GLP-1)

HbA1c - Hemoglobina glicada

IMC - Índice de massa corpórea

IHR - Índice de hiperemia reativa

NO - Nitric oxide

PA - Pressão Arterial

PSc - Pressão Arterial Sistólica Central

PAT - Peripheral arterial tonometry

PD - Pressão Diastólica

PS - Pressão Sistólica

PWV - Pulse wave velocity

RHI - Reactive hyperemia index

VMF - Vasodilatação mediada pelo fluxo

VOP - Velocidade de onda de pulso

Resumo

Introdução: Vários estudos demonstraram que os inibidores da dipeptidyl peptidase-4 (DPP-4), usados no tratamento de pacientes portadores de diabetes mellitus do tipo 2 (DM 2), melhoraram a função endotelial. **Objetivos:** O presente estudo avaliou os efeitos da vildagliptina, um inibidor de DPP-4, comparado à glibenclamida (sulfonilureia), na função endotelial, na rigidez arterial e na pressão arterial de 24 horas de pacientes com DM 2 e hipertensão arterial (HA). **Casuística e Métodos:** Este foi um estudo prospectivo, randomizado, aberto, controlado por fármaco, que incluiu cinquenta pacientes com idade superior a 35 anos, com DM 2 e HA, livres de doença cardiovascular, randomizados para tratamento com vildagliptina ou glibenclamida. Metformina foi adicionada a todos os pacientes. A monitorização ambulatorial de pressão arterial de 24 horas e avaliação da função endotelial foram realizadas antes e após 12 semanas de tratamento. A função endotelial foi avaliada pela tonometria arterial periférica (Endo-PAT 2000), que calcula o índice de hiperemia reativa (IHR) e a rigidez arterial por meio do augmentation index (Aix@75). A rigidez arterial também foi avaliada por parâmetros do Aix@75, velocidade da onda de pulso (VOP) e pressão arterial sistólica central (PSc) por meio de monitorização ambulatorial de 24 horas usando Mobil-O-Graph® PWA. O desfecho primário foi variação do IHR após tratamento com vildalgiptina comparado ao tratamento com glibenclamida. **Resultados:** Não houve diferença no IHR no grupo da vildagliptina (antes 2,348 ± 0,5868; depois 2,2408 ± 0,6019, P = 0,742) ou no grupo da glibenclamida (antes 2,3636 ± 0,5163; depois 2,3375 ± 0,4996, P = 0,950) e entre os grupos (P = 0,5479). Similarmente, o tratamento com vildagliptina e glibenclamida não produziu efeitos no AIx@75 PAT (P = 0,696), na 24-hs: PSc (P = 0,274) e na VOP (P = 0,324). **Conclusões:** O tratamento durante 12 semanas com vildagliptina em pacientes portadores de DM 2 e HA não alterou a função endotelial e nem a rigidez arterial. Assim, este fármaco apresenta uma ação neutra na função vascular, confirmando sua segurança no tratamento de pacientes com doença cardiovascular.

Palavras-chave: 1. Inibidor de DPP-4; 2. Função endotelial; 3. Vildagliptina; 4. Diabetes mellitus do tipo 2; 5. Hipertensão arterial; 6. Rigidez arterial.

Abstract

Introduction: Several trials have shown that dipeptidyl peptidase-4 (DPP-4) inhibitors, used to treat patients with diabetes mellitus type 2 (T2DM), improve endothelial function. **Objectives**: The current study investigated the effects of vildagliptin, a DPP-4 inhibitor, compared to glibenclamide on endothelial function and arterial stiffness (AS) in patients with T2DM and hypertension (HT). **Casuistics and Methods**: This trial was a prospective randomized, open label, controlled by drug. Fifty patients aged over 35 years with T2DM and hypertension, without cardiovascular disease, were randomly allocated to treatment with vildagliptin (n=25) or glibenclamide (n=25). Both groups used metformin. A 24-h non-invasive ambulatory blood pressure monitoring and assessment of endothelial function were performed before and after 12 weeks of treatment. Endothelial function was evaluated by peripheral artery tonometry (Endo-PAT 2000), measuring the reactive hyperemia index (RHI) and arterial stiffness. AS was also evaluated by augmentation index (Aix@75), pulse wave velocity (PWV) and central systolic blood pressure (cSBP) parameters with a portable compact digital BP recorder Mobil-O-Graph® 24-hour PWA monitor. The primary study outcome was change in the RHI after vildagliptin vs. glibenclamide treatment. **Results:** There were no changes in RHI in the vildalgliptin group (before 2.348 ± 0.5868; after 2.2408 ± 0.6019, P = 0.742) or in the glibenclamide group (before 2.3636 ± 0.5163; after 2.3375 ± 0.4996, P = 0.950) and no difference between groups (P = 0.5479). There was no difference between vildagliptin and glibenclamide treatment in AIx@75 PAT (P = 0.696), in 24-hs: cSBP (P = 0.274) and in PWV (P = 0.324). **Conclusions**: Vildagliptin in patients with T2DM and HT did not change endothelial function and AS during 12 weeks. Thus, this drug has a neutral effect on vascular function, providing its effectiveness for the treatment of patients with cardiovascular disease.

Key-Words*:* 1. DPP-4 inhibitor; 2. Endothelial function; 3 Vildagliptin; 4. Diabetes mellitus type 2; 5. Hypertension; 6. Arterial stiffness.

1. INTRODUÇÃO

1. INTRODUÇÃO

A doença cardiovascular (DCV) é a principal causa de mortalidade em países desenvolvidos e em desenvolvimento. No Brasil, a DCV é responsável por mais de 30% da mortalidade total e responde por 1,2 milhões de hospitalizações/ano.[1] Hipertensão arterial (HA) e diabetes mellitus do tipo 2 (DM 2) estão entre as principais causas de DCV, sendo a HA a mais prevalente das DCV, afetando 30-40% dos adultos (mais de 70 milhões de Americanos e 36 milhões de Brasileiros).[2-3] Por outro lado, 13,5 a 15% da população brasileira apresenta DM 2.[4-5] Com o envelhecimento da população global, é esperado que a prevalência de DM na população com idade entre 20 e 79 anos aumente de 8,8%, em 2015, para 10,4%, em 2040, em todo o mundo. Assim, é previsto um aumento proporcional da prevalência de HA e DM 2.[6]

O DM 2 está associado a um risco duas vezes maior de DCV.[7] A disfunção endotelial é um preditor independente para DCV em pacientes com DM 2[8] e, também é considerada um marcador precoce de complicacões vasculares,[9] além de estar envolvida no processo aterogênico da doença arterial coronariana (DAC).[10-11] Vários mecanismos estão envolvidos na disfunção endotelial do diabetes, entre eles: redução da biodisponibilidade do óxido nítrico, diminuição da vasodilatação dependente do endotélio, maior atividade inflamatória e estado pró-coagulante.[12]

No tratamento do DM 2, é importante o uso de fármacos que, além de melhorarem o controle glicêmico, promovam proteção cardiovascular. Recentemente, os fármacos que agem no sistema incretina parecem desempenhar efeitos benéficos na função endotelial.[13] Neste sentido, as incretinas *glucagon-like peptide-1* (GLP-1) e *glucose-dependent insulinotropic polypeptide* (GIP) pertencem a um grupo de hormônios gastrointestinais que estimulam a secreção de insulina em resposta à ingestão alimentar.

No entanto, as incretinas são rapidamente degradadas por uma enzima denominada dipeptidil-peptidase-4 (DPP-4) e, consequentemente, transformadas em metabólitos inativos.[13] Estudos demonstram que existe sinalização fisiológica do GLP-1 nas células endoteliais e nas células do músculo liso vascular, que promove uma ação vasodilatadora mediada por receptores específicos de GLP-1 no endotélio vascular e melhora a função endotelial.[14-16] Entretanto, os efeitos cardiovasculares do GLP-1 podem ser independentes de receptores e parecem atuar por meio de metabólitos do GLP-1.[17]

Várias pesquisas sobre a ação vascular dos agonistas/análogos do GLP-1 e inibidores de DPP-4 foram realizadas e os resultados são controversos.[18-22] Estudo com a exenatide, um agonista do GLP-1, mostrou um significante aumento da vasodilatação mediada pelo fluxo (VMF).[18] Adicionalmente, a liraglutida, análogo do GLP-1, reduziu os níveis do inibidor do ativador de plasminogênio 1 (PAI-1) e da arginina di-metil assimétrica, melhorando a biodisponibilidade do óxido nítrico.[19]

Outros estudos demonstraram um aumento do fluxo sanguíneo global do miocárdio, após a infusão do agonista de GLP-1 em pacientes com DM 2[20] e, também com a infusão de GLP-1 em indivíduos saudáveis.[21] Porém, a liraglutida não melhorou a função microvascular coronariana de pacientes com DM 2 em outro estudo.[22]

Os estudos realizados com inibidores de DPP-4, grupo de fármacos utilizados no tratamento do DM 2 que aumenta a meia-vida do GLP-1 endógeno, também mostraram resultados conflitantes em relação à função vascular. Os inibidores de DPP-4 disponíveis para a prática clínica são: sitagliptina, vildagliptina, saxagliptina, linaglipitna e alogliptina. Pesquisa realizada com a sitagliptina aumentou o número de células progenitoras endoteliais em pacientes portadores de DM 2.[23] Outro estudo com

a vildagliptina demonstrou melhora da vasodilatação dependente de endotélio, avaliando de forma invasiva o fluxo sanguíneo no antebraço.[24] Contrastando com esses resultados positivos, outras pesquisas demonstraram que a sitagliptina não alterou a função endothelial, após 12 e 24 meses de tratamento,[25] e houve piora da função endotelial com a sitagliptina e a alogliptina em outro estudo.[26]

Recentemente, três grandes estudos randomizados multicêntricos, que avaliaram a saxagliptina,[27] a alogliptina,[28] e a sitagliptina[29] não demonstraram nenhuma redução nos eventos cardiovasculares. Sobretudo, esses fármacos não aumentaram os desfechos primários e foram considerados seguros no tratamento de pacientes com DCV. Entretanto, houve um aumento do número de hospitalizações por insuficiência cardíaca no braço da saxagliptina do estudo SAVOR.[27]

Atualmente, vários métodos são utilizados para avaliar a função endotelial, incluindo os marcadores plasmáticos de atividade endotelial, avaliação da estrutura vascular como a medida da espessura da camada íntima-média da carótida, além da rigidez arterial, VMF e tonometria arterial periférica (*peripheral arterial tonometry, PAT*).

A avaliação da função endotelial com PAT (Endo-PAT 2000) em pacientes com DAC mostrou forte correlação deste método com a disfunção endotelial.[30-33] Por meio de uma técnica simples, não-invasiva, observador-independente e reprodutível, o método mede as alterações de volume no pulso digital antes e, após a oclusão do fluxo sanguíneo e calcula automaticamente o índice de hiperemia reativa (IHR), considerado um índice de função endotelial. Adicionalmente, a rigidez arterial é avaliada por meio do cálculo do índice de incremento *(augmentation index, AIx).* Entretanto, o método de PAT é diferente do VMF, pois nele se avalia a artéria de resistência enquanto no VMF o

alvo vascular é a artéria braquial, que é condutora. Embora a vasodilatação dependente do óxido nítrico endotelial seja mais proeminente com a técnica do VMF, e este método seja considerado o padrão-ouro na avaliação da função endotelial periférica, o Endo-PAT também reflete esse efeito.[34] Recentemente, uma meta-análise mostrou que ambos os métodos Endo-PAT e VMF são preditores para DCV; porém, mais estudos são necessários para determinar se os métodos são independentes um do outro.[35]

Outro parâmetro utilizado na investigação da disfunção endotelial é a rigidez arterial, reconhecida como marcador de risco para DCV,[36] de forma que a função endotelial alterada é um dos mecanismos envolvidos na patogênese da rigidez arterial.[37] A coexistência de HA e diabetes no paciente aumenta a rigidez arterial quando comparada à presença isolada de HA ou de DM 2.[38] Além disso, os parâmetros avaliados de rigidez arterial estão envolvidos em vários desfechos clínicos (DAC, acidente vascular cerebral, albuminúria, progressão da doença renal crônica, sobrevida de paciente com doença renal em estágio final e risco cardiovascular global)[39-44] e são mais importantes no prognóstico clínico em relação aos fatores de risco cardiovasculares clássicos conhecidos como idade, gênero, tabagismo e dislipidemia.[45]

Para avaliar a rigidez arterial, a tonometria de aplanação da artéria radial é um método não-invasivo que estima a complacência arterial, a pressão arterial (PA) central e também calcula o AIx, derivado da análise da onda de pressão da aorta.[46-47] A elevação do AIx correlaciona-se com uma maior rigidez arterial, contribuindo para o risco cardiovascular.[39,45] Existem evidências de que a medida da PA central (medida na raiz da aorta), representa um melhor fator preditivo de desfecho cardiovascular do que a medida da PA periférica (braquial).[48-50] Como AIx é influenciado pela frequência cardíaca, o índice corrigido pela frequência cardíaca de 75 batimentos por

minuto (AIx@75) é usado, conforme previamente descrito.[51] Além do AIx@75, a rigidez arterial também pode ser avaliada por parâmetros como pressão sistólica central, pressão de pulso e velocidade da onda de pulso (VOP).[52] Marcadores inflamatórios estão associados com a VOP em pacientes hipertensos resistentes, indicando correlação entre inflamação e rigidez arterial.[53]

Assim, o objetivo desse estudo foi avaliar o efeito da vildagliptina na melhora da função endotelial e da rigidez arterial, além dos efeitos benéficos deste fármaco no controle glicêmico. Para este propósito, esta pesquisa é apresentada em uma sequência de três artigos, que abordam o uso do fármaco vildagliptina em pacientes com DM 2 e HA.

Os dois primeiros artigos foram publicados em revistas indexadas e com fator de impacto. O primeiro artigo refere-se a um relato de caso, no qual foi utilizada a tonometria de aplanação da artéria radial para explorar o efeito da vildagliptina na rigidez arterial, ao avaliar a PA central e o AIx@75. O segundo artigo trata do protocolo de pesquisa dos efeitos da vildagliptina na função endotelial, na rigidez arterial e na monitorização da pressão arterial de 24 horas (PA central, PA periférica, VOP, pressão de pulso) em pacientes com DM 2 e HA. Para essa finalidade a vildagliptina foi comparada à glibenclamida, que pertence ao grupo das sulfonilureias sem ação benéfica vascular comprovada,[54] sendo que ambas foram associadas à metformina. O terceiro artigo mostra os resultados do protocolo de pesquisa relatado no segundo artigo. Para realização da pesquisa, foram utilizados os aparelhos Endo-PAT 2000 e aparelho de PA digital de 24 horas Mobil-O-Graph® PWA.

2. RESULTADOS

2. RESULTADOS

2.1. Artigos Científicos

Os resultados deste trabalho encontram-se descritos em dois artigos submetidos e um a ser sumetido à publicação em revistas indexadas.

Artigos:

1. **Luciana Neves Cosenso-Martin**, Luiz Tadeu Giollo-Júnior, José Fernando Vilela-Martin. **DPP-4 Inhibitor Reduces Central Blood Pressure in a Diabetic and Hypertensive Patient: A Case Report.** (Publicado na revista **Medicine (Baltimore). 2015 Jul;94(27):e1068./** doi: 10.1097/MD.0000000000001068).

2. **Luciana Neves Cosenso-Martin,** Luiz Tadeu Giollo-Júnior, Débora Dada Martineli, Cláudia Bernardi Cesarino, Marcelo Arruda Nakazone, José Paulo Cipullo, José Fernando Vilela-Martin. Twelve-week randomized study to compare the efect of vildagliptin vs. glibenclamide both added-on to metformin on endothelium function in patients with type 2 diabetes and hypertension. (Publicado na revista **Diabetology and Metabolic Syndrome. 2015 Aug 26;7:70./** doi: 10.1186/s13098-015-0062-z).

3. **Luciana Neves Cosenso-Martin,** Luiz Tadeu Giollo-Júnior, Marcelo Arruda Nakazone, José Fernando Vilela-Martin. The effect of vildagliptin on endothelial function, arterial stiffness and blood pressure in patients with type 2 diabetes and hypertension. (A ser submetido para revista **Diabetes Care**).

2.1.1 ARTIGO 1

2.1.1 ARTIGO 1

Medicine®
CLINICAL CASE REPORT

OPEN

DPP-4 Inhibitor Reduces Central Blood Pressure in a Diabetic and Hypertensive Patient

A Case Report

Luciana Neves Cosenso-Martin, MD, Luiz Tadeu Giollo-Junior, MSc, and José Fernando Vilela-Martin, MD, PhD, FAHA

Abstract: Hypertension and type 2 diabetes mellitus (DM) are among the main risk factors for the development of cardiovascular disease. Pharmacotherapy for DM should not only improve blood glucose control, but also provide beneficial glucose-independent cardiovascular effects. The central systolic blood pressure (SBP) has become more important than the brachial SBP in the assessment of cardiovascular risk.

This case report describes the effect of vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, on the central SBP in a 54-year-old woman with hypertension and DM. She was submitted to applanation tonometry (AT) before and after vildagliptin association. AT of the radial artery is a non-invasive method that indirectly assesses arterial stiffness by calculating the central SBP and the augmentation index (AIx).

After 3 months of follow-up using vildagliptin, central SBP and AIx were improved. Moreover, she presented better glycemic control.

This case suggests an effect of DPP-4 inhibitor on arterial stiffness parameter (central SBP) in a hypertensive and diabetic patient, which shows a glucose-independent beneficial cardiovascular effect of this group of drugs.

(*Medicine* 94(27):e1068)

Abbreviations: AIx = augmentation index, AIx75 = adjusted augmentation index for a heart rate of 75 bpm, AT = applanation tonometry, BMI = body mass index, BP = blood pressure, CHD = coronary heart disease, cIMTcarotid = intima-media thickness, CVD = cardiovascular disease, DM = diabetes mellitus, DPP-4 = dipeptidyl peptidase-4, GLP-1 = glucagon-like peptide 1, HbA1c = glycosylated hemoglobin, NO = nitric oxide, SBP = systolic blood pressure, TOD = target organ damage.

Editor: Asim Ahmed Elnour.
Received: February 24, 2015; revised: May 20, 2015; accepted: May 21, 2015.
From the Department of Internal Medicine, Medical School of São José do Rio Preto (FAMERP), Hypertension Clinic of FAMERP and Hospital de Base, Ave Brig Faria Lima 5416, São José do Rio Preto, SP, Brazil.
Correspondence: José Fernando Vilela-Martin, Department of Internal Medicine, Medical School of São José do Rio Preto (FAMERP), Hypertension Clinic of FAMERP and Hospital de Base, Ave Anísio Haddad 7700, São José do Rio Preto, SP 15093-000, Brazil (e-mail: vilelamartin@uol.com.br).
LNC-M, LTG-J, and JFV-M contributed equally to the literature search, data interpretation, figure creation, and writing of the manuscript. They have read and approved the final manuscript.
JFV-M has received research grants from Novartis. However, it has no role in study design, data collection and analysis, decision to publish, or preparation of the article.
LNC-M and LTG-J have indicated that they have no conflicts of interest regarding the content of this article.
Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
This is an open access article distributed under the Creative Commons Attribution License 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001068

INTRODUCTION

Hypertension and type 2 diabetes mellitus (DM) are among the main risk factors for the development of cardiovascular disease (CVD). In fact, DM is associated with a 2 fold higher risk for CVD.[1] Endothelial dysfunction, associated with DM and hypertension, is considered an early marker of vascular complications and a pathophysiological determinant of atherogenic processes.[2] Pharmacotherapy for DM should not only improve blood glucose control, but also provide beneficial glucose-independent cardiovascular effects. Dipeptidyl peptidase-4 (DPP-4) inhibitor is an incretin-based drug approved for the treatment of DM.[3] This medication reduces the breakdown of glucagon-like peptide 1 (GLP-1), thereby increasing circulating GLP-1 levels, improving metabolic control by increases in insulin secretion, followed by decreases in glucagon secretion.[3] Pharmacotherapy based on the GLP-1 system provides beneficial effects on the endothelium.[4–6] Recently, several methods have been developed to assess endothelial function, and predict the presence or absence of coronary heart disease (CHD).[7] Applanation tonometry (AT) of the radial artery is a noninvasive method that indirectly assesses arterial stiffness by calculating the central blood pressure (BP) and the augmentation index (AIx).[8,9] The AIx is associated with cardiovascular risk, and is a predictor of CHD development.[9] More recently, the central systolic blood pressure (SBP) of the aortic or carotid arteries has become more important than the brachial SBP in the assessment of cardiovascular risk.[10] This case report describes a possible pleiotropic action of a DPP-4 inhibitor (vildagliptin) on the central SBP assessed by AT in a hypertensive diabetic woman. This pharmacological class seems to have action in reduction of central BP and arterial stiffness. Thus, we justify the possible pathophysiological mechanisms involved in the association between hyperglycemia, endothelial dysfunction, and vascular stiffness, besides how the GLP-1 system provides beneficial effect on the endothelium.

CASE REPORT

The patient was a 54-year-old white woman with a 4-year history of hypertension and DM. She was taking metformin (850 mg/d), pioglitazone (30 mg/d), simvastatin (10 mg/d), amlodipin (5 mg/d), and enalapril (10 mg/d); however, she did not adhere to a diet to control the diabetes. Her physical examination revealed BP: 123/85 mm Hg, heart rate: 78 bpm, body mass index (BMI): 29.1 kg/m^2, and waist circumference: 91 cm. She had no abnormalities of the heart, lungs, or abdomen. According to complementary exams, the patient had poor diabetic control with glycosylated hemoglobin (HbA1c): 11.2%; however, microalbuminuria and other biochemical parameters were normal.

The patient received guidance to modify her lifestyle including diet and exercise, and vildagliptin (100 mg/d) was

added to her drug regimen. The patient was submitted to examinations of the radial artery using a commercially available automated AT system (HEM-9000AI; Omron Healthcare Co., Ltd, Kyoto, Japan) before receiving vildagliptin and 3 months after to evaluate the drug's effect on the central SBP and AIx. This examination was performed in a quiet controlled environment (temperature between 21°C to 24°C), between 8 AM and 10 AM, after the patient was rested for at least 10 minutes sitting with the legs uncrossed, the bladder empty, and away from acute stressors.[9] All measurements were performed after at least 8-hour fasting. The patient was instructed to fast starting the night before testing and to refrain from ingesting alcohol or caffeine. The AT equipment uses a radial ultrasonic transducer and cuffs with the correct size for the arm circumference as recommended by the guidelines to evaluate BP. Pulse wave analyses were performed at least 3 times and the mean of measurements was calculated. Variations of BP between the measurements should not be >5%.[9]

At the end of the 3-month follow-up period, the patient had good adherence to diet and exercise and had lost 3 kg, presenting with a BMI of 27.5 kg/m^2, and an office BP of 100/70 mm Hg. The control of the diabetes was better with a level of HbA1c of 7.2%. Central SBP and AIx were lower than in the baseline results. Before the association of vildagliptin and metformin, the peripheral SBP was 129 mm Hg during the AT. The central SBP (aortic) and adjusted AIx for a heart rate of 75 bpm (AIx75) were 127 mm Hg and 96%, respectively (Figure 1A). After receiving vildagliptin for 3 months, the peripheral SBP was 116 mm Hg, central SBP was 101 mm Hg, and AIx75 was 72% (Figure 1B) during the AT. So, there were reductions in both the central SBP and AIx with central SBP becoming 15 mm Hg lower than the peripheral SBP. I affirm that the patient has given the informed consent for publication of the case.

DISCUSSION

DM is associated with a 2 fold higher risk for CVD.[1] Preventive strategies are important to reduce cardiovascular risk. An assessment and stratification of cardiovascular risk should be considered in order to identify individuals at higher risk of developing cardiovascular events. Thus, the adoption of preventive and pharmacological strategies is important to delay possible cardiovascular complications. In recent years, several methods have been developed to predict cardiovascular risk, including the use of ultrasound to measure the carotid intima-media thickness (cIMT) and the aortic pulse wave velocity, and computed tomography to identify and quantify calcification of coronary arteries, among others.[7]

In this case, the AT of the radial artery was used to assess vascular disease. In young healthy individuals, the central SBP (aortic) should be between 15 and 20 mm Hg lower than the peripheral SBP (brachial). Before DPP-4 inhibitor use in this patient, the central SBP was only 2 mm Hg lower than the peripheral SBP. After 3 months in use of vildagliptin, the difference between the central SBP and peripheral SBP increased to 15 mm Hg. This finding might be explained by the effect of the drug on the arterial tree, that is, it may represent a reduction in arterial stiffness. The central SBP and AIx are strongly correlated. In this case, AIx also improved after DPP-4 inhibitor use, even though both were normal at baseline. AIx is associated with cardiovascular risk as it identifies the presence or absence of CHD, and thus it is also considered a marker of vascular stiffness.[9]

According to European Society of Hypertension Guidelines, the evaluation of asymptomatic target organ damage (TOD) is crucial in determining the cardiovascular risk in hypertensive individuals. Among the TOD to be assessed is vascular stiffness, which can predict cardiovascular mortality independently of the stratification scores of cardiovascular risk.[11] The evaluation of central hemodynamic, including carotid-femoral pulse wave velocity, central BP, and AIx, is important to determine the true cardiovascular risk. The Guidelines state that the measurement of central hemodynamic parameters is of great interest to mechanistic analyses in pathophysiology, pharmacology, and therapeutics, and that the central BP represents the true load imposed on heart, brain, kidney, and large arteries instead of the brachial BP.[11] Thus, both central BP and AIx present better predictive value for

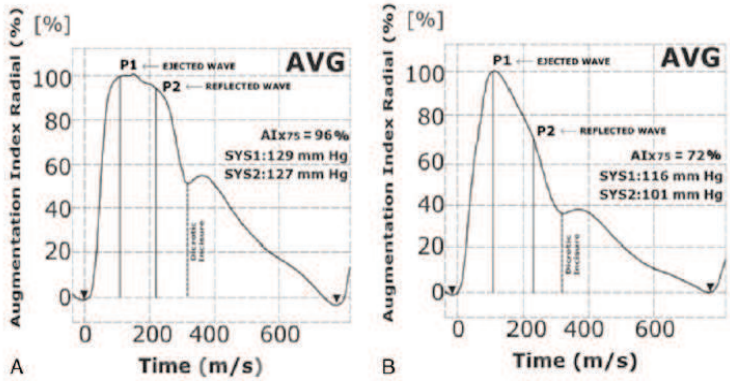

FIGURE 1. Waves and values of blood pressure measured with the applanation tonometry. (A) Before association of vildagliptin and metformin, Sys1 was 129 mm Hg. Sys2 and adjusted AIx for a heart rate of 75 bpm (AIx75) were 127 mm Hg e 96%, respectively. AIx75 under 100% is considered normal. Sys2 (central) was only 2 mm Hg lower than the peripheral systolic blood pressure and this difference should be about 15 to 20 mm Hg or 15% lower than Sys1. (B) After 3 months of vildagliptin and metformin association, Sys1 was 116 mm Hg, Sys2 was 101 mm Hg, and AIx75 was 72%. Both Sys2 and AIx presented reduction and Sys2 had become 15 mm Hg lower than the Sys1. AIx = augmentation index, AIx75 = adjusted augmentation index for a heart rate of 75 bpm, Sys1 = peripheral systolic blood pressure, Sys2 = central systolic blood pressure.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

cardiovascular events and for the differential effect of antihypertensive drugs compared to the brachial BP.[11,12]

Endothelial dysfunction is considered an early marker of vascular complications and a pathophysiological determinant of the atherogenesis that occurs in the early stages of CHD.[2] Exposure to cardiovascular risk factors may trigger the atherosclerotic process that evolves with oxidative stress and nitric oxide (NO) inactivation.[13] Endothelial dysfunction comprises a number of functional alterations such as impaired endothelium-dependent vasodilatation, impaired barrier function, and higher inflammatory and pro-coagulant activity.[13]

Although pharmacotherapy based on the GLP-1 system may provide beneficial effects to the endothelium (Figure 2), there are no studies on the central BP. Different methodologies have demonstrated the effect of this pharmacological class on endothelium.[4–6,14,15] Recently, vildagliptin improved endothelium-dependent vasodilatation in subjects with DM.[5] However, this study included an invasive method to assess endothelial function, which is not applicable in the clinical practice.[5] Sitagliptin, another DPP-4 inhibitor, demonstrated the same beneficial effects: an increase in endothelial progenitor cells by inhibiting the degradation of the chemokine stromal-derived factor 1-alpha[4] and improved endothelial function in uncontrolled diabetic patients.[6] Some studies on this class of drugs have shown effects on NO modulation.[6,14] Liraglutide, a new GLP-1 receptor agonist, reduced the plasminogen activator inhibitor 1 and asymmetric dimethylarginine levels and, consequently, improved NO availability.[15]

Several mechanisms may be underlying the improvement in endothelium function linked to vildagliptin use. Firstly, a vasodilator response to acetylcholine was observed in the vascular bed.[5] In second place, vildagliptin is able to control the daily acute glucose fluctuations and delay the progression of atherosclerosis in DM.[16] In this study, it was demonstrated that the cIMT, a surrogate of carotid atherosclerosis, decreased 3 months after the use of both vildagliptin and sitagliptin. Finally, GLP-1 has been shown to exert anti-inflammatory effects on different tissues and to decrease daily oxidative stress parameters.[17] Thus, the decrease in the cIMT might be mediated by improved vascular inflammation and endothelial dysfunction. In the present study, daily acute glucose fluctuations were not evaluated.

To better differentiate the effect of DPP-4 inhibitors on hemodynamic parameters (endothelial function, arterial stiffness, peripheral, and central SBP), it is important to consider the role played by changes in lifestyle on these benchmarks. Some authors showed that individuals treated with DPP-4 inhibitors presented reductions in peripheral SBP independent of decreases in blood glucose and without reducing BMI.[18] On the contrary, in obese patients with type 2 diabetes, treatment using DPP-4 inhibitors in combination with metformin was associated with improvements in glycemic control and a reduction in body weight.[19] Thus, it remains to be seen whether the reduction in BP with DPP-4 inhibitor treatment is related to the improvements in blood glucose and the drop in body weight or the effects of this therapeutic drug itself or both.

The effect of weight loss on central BP is controversial. Recently, a study showed that weight loss was significantly and independently associated with central BP and brachial BP. However, the study population included a small number of hypertensive (35.8%) and diabetic (15.8%) patients.[20] In morbidly obese dysglycemic subjects without hypertension, modest weight loss reduced arterial stiffness.[21] Another study demonstrated that 10.6% of weight loss did not influence the vascular stiffness in a nondiabetic population.[22] In obese children and adolescents, no clear effect on arterial stiffness was found in respect to weight reduction.[23] Moreover, low-fat versus low-carbohydrate diet in adults without DM demonstrated

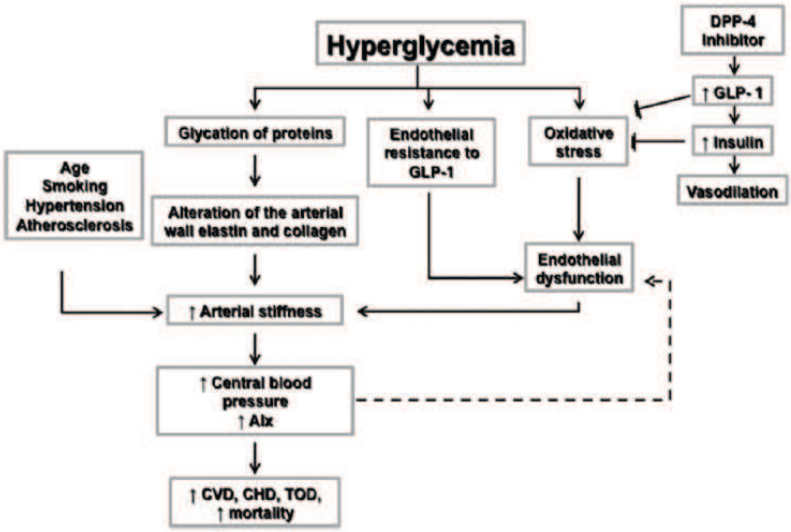

FIGURE 2. Pathophysiological mechanisms that associate hyperglycemia, endothelial dysfunction, arterial stiffness, and cardiovascular disease. This figure explains how the hyperglycemia causes endothelial damage, which results in arterial stiffness and increased central BP. The GLP-1 system provides beneficial effect on the endothelium in 2 ways, directly in the oxidative stress modulation (blocked arrow) or indirectly in the insulin production increase. The elevated central BP and AIx may interfere in the endothelial dysfunction (dashed arrow), and contribute to the arterial stiffness. AIx = augmentation index, BP = blood pressure, CHD = coronary heart disease, CVD = cardio-cardiovascular disease, DPP-4 = dipeptidyl peptidase-4, GLP-1 = glucagon-like peptide 1, TOD = target organ damage.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

significant weight loss in both groups. However, arterial stiffness improved only in the group following the low-fat diet.[24] In conclusion, with these data, the effect of weight loss on arterial stiffness is not clear, suggesting that more studies are necessary, including a study with a specific population (hypertensive and DM without coronary disease). In relationship to exercise, there is evidence that resistance training has an effect on the central BP[25]; however, the patient in this case did not do intense exercising. In respect to HbA1c, some studies demonstrated weight loss in adults with DM managed by low-carbohydrate diet, but the HbA1c level decreased only by 0.6% to 1.0%, while the weight loss was between 1.2 and 4.2 kg. These studies justified that weight loss participated in glycemic control, but it was not enough to decrease the HbA1c level.[26,27]

Furthermore, studies have shown solely reduction in peripheral BP with the use of DPP-4 inhibitors, without observing the effects on the central BP. So, we strongly believe that vildagliptin was the main responsible for the improvement in endothelial function, and peripheral and central SBP. Anyway, as we know that one case report is not proof of the effect of DPP-4 inhibitor on central BP, a study that evaluates the endothelial function and glucose-independent beneficial cardiovascular effects of DPP-4 inhibitor is being carried out with this purpose. (J.F. Vilela-Martin, MD, PhD, unpublished data, February 2015, https://clinicaltrials.gov/ct2/show/NCT02145611?term=vildagliptin&rank=8).

CONCLUSION

In conclusion, to the best of our knowledge this is the first report that suggests an effect of the DPP-4 inhibitor on arterial stiffness parameter (central BP) in a hypertensive and type 2 diabetic individual, using a noninvasive method for evaluating the central BP. Other studies that evaluate the endothelial function and show glucose-independent beneficial cardiovascular effects of DPP-4 inhibitor are expected.

ACKNOWLEDGMENTS

The authors thank the reviewer for correcting both spelling and grammar of the English text.

REFERENCES

1. Emerging Risk Factors CollaborationSarwar N, Gao P, Seshasai SR, et al. . Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* 2010;375:2215–2222.
2. Perrone-Filardi P, Cuocolo A, Brevetti G, et al. Relation of brachial artery flow-mediated vasodilation to significant coronary artery disease in patients with peripheral arterial disease. *Am J Cardiol.* 2005;96:1337–1341.
3. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet.* 2006;368:1696–1705.
4. Fadini GP, Boscaro E, Albiero M, et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal derived factor-1 alpha. *Diabetes Care.* 2010;33:1607–1609.
5. van Poppel PC, Netea MG, Smits P, et al. Vildagliptin improves endothelium-dependent vasodilation in type 2 diabetes. *Diabetes Care.* 2011;34:2072–2077.
6. Matsubara J, Sugiyama S, Akiyama E, et al. Dipeptidyl peptidase-4 inhibitor, sitagliptin, improves endothelial dysfunction in association with its anti-inflammatory effects in patients with coronary artery disease and uncontrolled diabetes. *Circ J.* 2013;77:1337–1344.
7. Tousoulis D, Antoniades C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart.* 2005;91:553–558.
8. DeLoach SS, Townsend RR. Vascular stiffness: its measurement and significance for epidemiologic and outcome studies. *Clin J Am Soc Nephrol.* 2008;3:184–192.
9. Patvardhan E, Heffernan KS, Ruan J, et al. Augmentation index derived from peripheral arterial tonometry correlates with cardiovascular risk factors. *Cardiol Res Pract.* 2011;2011:253758 (1–6).
10. Agabiti-Rosei E, Mancia G, O'Rourke MF, et al. Central blood pressure measurements and antihypertensive therapy: a consensus document. *Hypertension.* 2007;50:154–160.
11. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens.* 2013;31:1281–1357.
12. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation.* 2010;121:505–511.
13. Bakker W, Eringa EC, Sipkema P, et al. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell Tissue Res.* 2009;335:165–189.
14. Mason RP, Jacob RF, Kubant R, et al. Dipeptidyl peptidase-4 inhibition with saxagliptin enhanced nitric oxide release and reduced blood pressure and sICAM-1 levels in hypertensive rats. *J Cardiovasc Pharmacol.* 2012;60:467–473.
15. Forst T, Michelson G, Ratter F, et al. Addition of liraglutide in patients with Type 2 diabetes well controlled on metformin monotherapy improves several markers of vascular function. *Diabet Med.* 2012;29:1115–1118.
16. Barbieri M, Rizzo MR, Marfella R, et al. Decreased carotid atherosclerotic process by control of daily acute glucose fluctuations in diabetic patients treated by DPP-IV inhibitors. *Atherosclerosis.* 2013;227:349–354.
17. Lee YS, Park MS, Choung JS, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia.* 2012;55:2456–2468.
18. Ogawa S, Ishiki M, Nako K, et al. Sitagliptin, a dipeptidyl peptidase-4 inhibitor, decreases systolic blood pressure in Japanese hypertensive patients with type 2 diabetes. *Tohoku J Exp Med.* 2011;223:133–135.
19. Velija-Asimi Z, Izetbegovic S, Karamehic J, et al. The effects of dipeptidyl peptidase-4 inhibitors in treatment of obese patients with type 2 diabetes. *Med Arch.* 2013;67:365–367.
20. Kawamoto R, Kohara K, Katoh T, et al. Effect of weight loss on central systolic blood pressure in elderly community-dwelling persons. *Hypertens Res.* 2014;37:933–938.
21. Samaras K, Viardot A, Lee PN, et al. Reduced arterial stiffness after weight loss in obese type 2 diabetes and impaired glucose tolerance: the role of immune cell activation and insulin resistance. *Diab Vasc Dis Res.* 2013;10:40–48.
22. Olsen RH, Pedersen LR, Jürs A, et al. A randomised trial comparing the effect of exercise training and weight loss on microvascular function in coronary artery disease. *Int J Cardiol.* 2015;185:229–235.
23. Hvidt KN, Olsen MH, Ibsen H, et al. Weight reduction and aortic stiffness in obese children and adolescents: a 1-year follow-up study. *J Hum Hypertens.* 2015doi: 10.1038/jhh.2014.127. [Epub ahead of print].

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

24. Bradley U, Spence M, Courtney CH, et al. Low-fat versus low-carbohydrate weight reduction diets: effects on weight loss, insulin resistance, and cardiovascular risk: a randomized control trial. *Diabetes*. 2009;58:2741–2748.

25. Croymans DM, Krell SL, Oh CS, et al. Effects of resistance training on central blood pressure in obese young men. *J Hum Hypertens*. 2014;28:157–164.

26. Westman EC, Yancy WS Jr, Mavropoulos JC, et al. The effect of a low-carbohydrate, ketogenic diet versus a low-glycemic index diet on glycemic control in type 2 diabetes mellitus. *Nutr Metab (Lond)*. 2008;5:36 (1–9).

27. Haimoto H, Iwata M, Wakai K, et al. Long-term effects of a diet loosely restricting carbohydrates on HbA1c levels, BMI and tapering of sulfonylureas in type 2 diabetes: a 2-year follow-up study. *Diabetes Res Clin Pract*. 2008;79:350–356.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

2.1.2 ARTIGO 2

2.1.2 ARTIGO 2

Cosenso-Martin *et al. Diabetol Metab Syndr (2015) 7:70*
DOI 10.1186/s13098-015-0062-z

DIABETOLOGY & METABOLIC SYNDROME

RESEARCH Open Access

Twelve-week randomized study to compare the effect of vildagliptin vs. glibenclamide both added-on to metformin on endothelium function in patients with type 2 diabetes and hypertension

Luciana Neves Cosenso-Martin[1,2], Luiz Tadeu Giollo-Júnior[1,2], Débora Dada Martineli[1,2], Cláudia Bernardi Cesarino[1,2], Marcelo Arruda Nakazone[1,2], José Paulo Cipullo[1,2] and José Fernando Vilela-Martin[1,2*]

Abstract

Background: Vildagliptin, a DPP-4 inhibitor widely used for the treatment of type 2 diabetes mellitus (T2DM), shows beneficial effects on endothelial function. This study aims to evaluate the effect of vildagliptin on endothelial function and arterial stiffness in patients with T2DM and hypertension.

Methods: Fifty over 35-year-old patients with T2DM and hypertension, without cardiovascular disease, will be randomly allocated to two groups: group 1 will receive vildagliptin added-on to metformin and group 2, glibenclamide added-on to metformin. Biochemical tests (glycemia, glycated hemoglobin, total cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, alanine aminotransferase, ultrasensitive C-reactive protein, and microalbuminuria), 24-h non-invasive ambulatory blood pressure monitoring, and assessment of endothelial function and arterial stiffness will be performed in both groups before and after 12 weeks of treatment. The endothelial function will be assessed by peripheral arterial tonometry, which measures the reactive hyperemia index (vasodilation), and arterial stiffness will be evaluated by applanation tonometry. All analysis will be performed using SPSS Statistical Software. For all analysis, a 2-sided $P < 0.05$ will be considered statistically significant.

Results: The study started in December 2013 and patient recruitment is programed until October 2015. The expected results are that vildagliptin will improve the endothelial function in patients with T2DM and hypertension compared to glibenclamide treatment, independently of glycemic control.

Conclusions: It is expected that this DPP-4 inhibitor will improve endothelial function in patients with T2 DM.

Trial registration: Clinical Trials NCT02145611, registered on 11 Jun 2013

Keywords: Type 2 diabetes mellitus, Hypertension, Endothelial dysfunction, Arterial stiffness, DPP-4 inhibitor

Background

Cardiovascular disease (CVD) is the main cause of deaths in developing and developed countries. In Brazil, CVD accounts for more than 30 % of the overall mortality rate and is responsible for 1.2 million hospitalizations/year [1]. Hypertension (HT) and type 2 diabetes mellitus (T2DM) are among the main causes of CVD. Hypertension is the most prevalent of all CVD affecting about 30–40 % of adults (over 70 million Americans and 36 million Brazilians) [2, 3]. The prevalence of T2DM ranges

*Correspondence: vilelamartin@uol.com.br
[2] Internal Medicine Department and Hypertension Clinic, State Medical School in São José do Rio Preto (FAMERP), Ave Brig. Faria Lima 5416, São José do Rio Preto, SP 15090-000, Brazil
Full list of author information is available at the end of the article

BioMed Central

© 2015 Cosenso-Martin et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

from 13.5 to 15 % of the population [4, 5]. As the number of elderly population is continuously growing around the world, in the next two to three decades there will be a 200 % increase in the number of individuals with ages >65 years. The prevalence of HT and T2DM is expected to increase proportionately [6].

T2DM is associated with a twofold higher risk for CVD [7]. Endothelial dysfunction is an independent predictor for future CVD in patients with T2DM [8] and is considered an early marker of vascular complications [9]. It is involved in the atherogenesis that occurs in the early stages of coronary artery disease (CAD) [10, 11]. The mechanisms of impaired endothelial function in diabetes are: reduced bioavailability of nitric oxide (NO), diminished endothelium-dependent vasodilatation, impaired barrier function, inflammatory activation, and a procoagulant state [12].

Today, some groups of drugs that act on the incretin system, such as glucagon-like peptide-1 (GLP-1) analogues/agonists and dipeptidyl peptidase-4 enzyme (DPP-4) inhibitors, are used to treat T2DM and may be responsible for beneficial effects on endothelial function [13].

Incretins such as GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) belong a group of gastrointestinal hormones that stimulate insulin secretion in response to the ingestion of food [13]. There is evidence of physiological signaling by GLP-1 in endothelial and vascular smooth muscle cells [14–16]. GLP-1 has a vasodilator action mediated through a specific GLP-1 receptor in the vascular endothelium and may improve endothelial function in animals and humans [14, 16]. However, cardiovascular effects may be GLP-1 receptor-independent, and mediated by the metabolites of GLP-1 [17]. One study with exenatide, a GLP-1 agonist, showed a significant increase in flow-mediated vasodilatation (FMD) [18]. Moreover, liraglutide, a GLP-1 analogue, reduced plasminogen activator inhibitor 1 (PAI-1) and asymmetric dimethylarginine (ADMA) levels, thereby improving nitric oxide availability [19]. Other researchers found increases in global myocardial blood flow following GLP-1 agonist infusions in T2DM [20], and following GLP-1 infusions in healthy humans [21].

On the other hand, DPP-4 rapidly degrades incretin hormones to inactive metabolites [13] thus DPP-4 inhibitors may improve endothelial function. Studies with sitagliptin, a DPP-4 inhibitor, demonstrated an increase of endothelial progenitor cells in T2DM by inhibiting the degradation of the chemokine stromal-derived factor 1-α [22]. According to an invasive method used to measure forearm blood flow during acetylcholine infusion in T2DM patients, vildagliptin, another DPP-4 inhibitor, improved endothelium-dependent vasodilatation [23]. In contrast, other studies did not demonstrate beneficial effects in relation to the endothelium [24, 25].

Recently, three large multicenter, randomized trials testing saxagliptin [26], alogliptin [27], and sitagliptin [28] did not find any reduction in cardiovascular events, but these drugs did not increase the risk of the primary end-point either. However, the rate of hospitalization for heart failure increased in the saxagliptin arm of the SAVOR study [26]. Thus, the researchers concluded that GLP-1 inhibitors are safe for patients with CVD.

Several methods have been used to assess impaired endothelial function including plasma concentrations of markers of endothelial activity, vessel structure related to the carotid intima media thickness and arterial stiffness, flow-mediated vasodilation (FMD) and peripheral arterial tonometry (PAT).

PAT is a simple, non-invasive, and reproducible technique used to assess endothelial function [29]. Measurements of PAT in patients with CAD have been shown to strongly correlate with the parameters of endothelial dysfunction [30–33]. The Endo-PAT is an observer-independent technique that measures volume changes in the fingertip before and after blood flow occlusion and automatically calculates the reactive hyperemia index (RHI), providing an index for endothelial function.

Arterial stiffness is another parameter used to investigate endothelial dysfunction. It is recognized as a cardiovascular risk marker [34] as impaired endothelial function is one of the mechanisms involved in increased vascular stiffness [35]. Patients with both HT and T2DM exhibit increased arterial stiffness compared to those with either T2DM or HT alone [36]. Arterial stiffness parameters predict clinical outcomes (CAD, stroke, urinary albumin excretion, progression of chronic kidney disease, survival in end-stage renal disease and general cardiovascular risk) [37–42] and have a greater importance in clinical prognoses compared to other known cardiovascular risk factors such as age, gender, smoking, and dyslipidemia [43]. The non-invasive applanation tonometry technique assesses arterial stiffness by estimating arterial compliance and central blood pressure (BP) and calculates the augmentation index (AIx) [44–46]. The AIx is a marker of wave reflection derived from aortic pressure wave analysis, with increased AIx being correlated to increased stiffness and contributing to cardiovascular risk [37, 43]. Evidence shows that the central blood pressure is more relevant to cardiovascular outcomes than the BP in the brachial artery [47–49]. More recently, a study demonstrated improvement in central BP and AIx following the use of vildagliptin in a patient with T2DM and HT [50].

Thus, using the non-invasive Endo-PAT 2000 device and radial artery applanation tonometry, the purpose of

this study is to evaluate the effect of vildagliptin compared to glibenclamide both added-on to metformin on endothelial function and arterial stiffness in patients with T2DM and hypertension.

Methods

The present trial (clinicaltrials.gov identifier: NCT02145611) will be randomized, open label, parallel assignment, controlled by drug. It was designed to assess the effect of vildagliptin (100 mg/day b.i.d.) on endothelial function in patients with T2DM and hypertension compared to glibenclamide (5–20 mg/day depending on glycemic control). The Research Ethics Committee of the institution approved the study protocol according to national and international guidelines. All patients will give their informed consent. Twenty-five individuals with T2DM and hypertension will be evaluated in the vildagliptin plus metformin group compared to 25 diabetic and hypertensive subjects in the glibenclamide plus metformin group. Figure 1 shows a flow chart of participant selection and interventions. The inclusion and exclusion criteria are presented in Table 1.

Random allocation

A computer validated software (Random allocator) will be used for random allocation. The study coordinator will organize and number the envelopes which will be allocated in order of patient enrollment. The professional responsible for the Endo-PAT procedure and applanation tonometry of radial artery will be blinded.

Randomization and follow-up

After screening for eligibility, 50 individuals will be submitted to an evaluation of endothelial function using the Endo-PAT 2000 device and measurement of the arterial stiffness by applanation tonometry of radial artery. Subsequently, they will be randomly allocated to the two arms of the study: Group 1 will receive vildagliptin

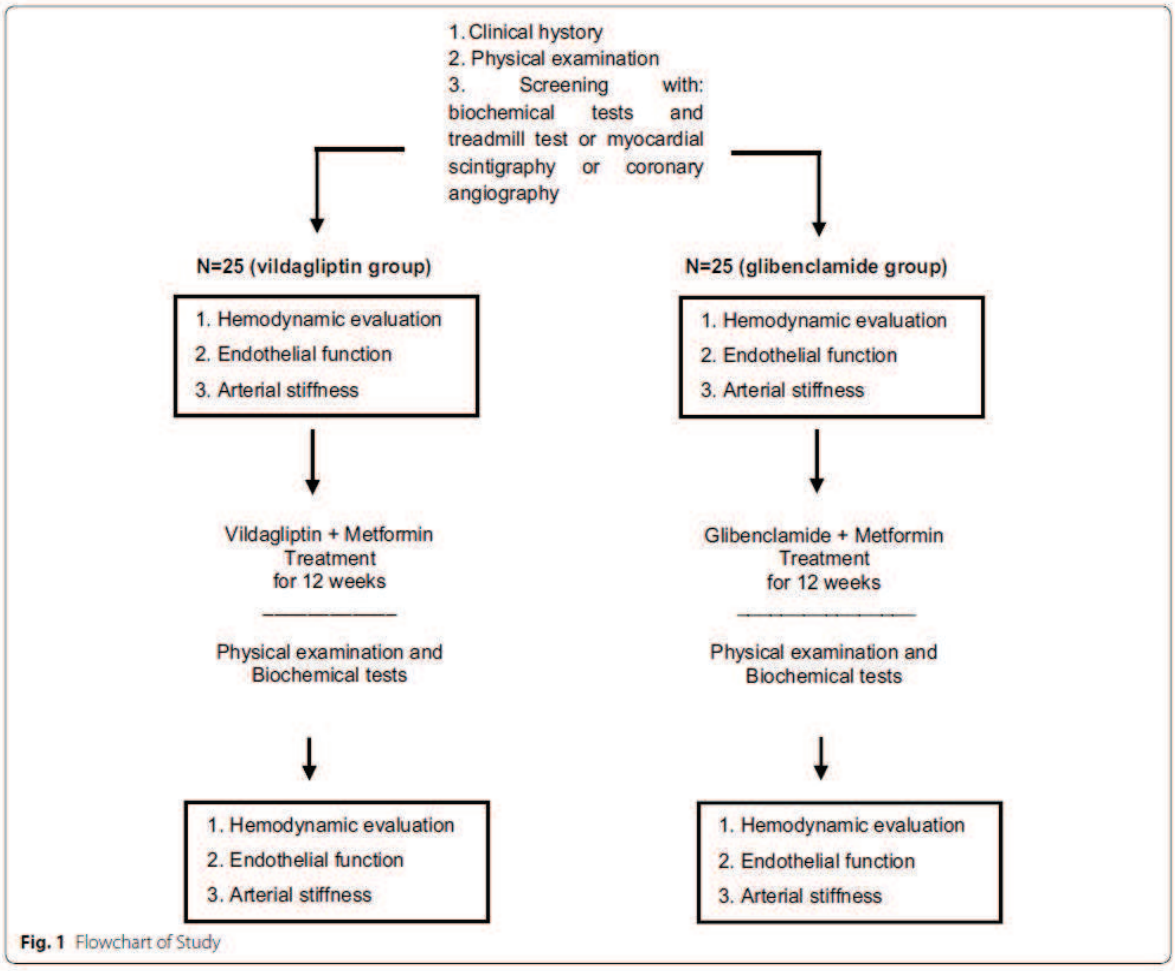

Fig. 1 Flowchart of Study

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Patients aged ≥35 years	Use of NPH or regular insulin, pioglitazone, GLP-1 receptor agonist, DPP-4 inhibitor or acarbose
History of DM and mild hypertension (blood pressure <160/100 mmHg) no longer than 15 years	Intolerance to metformin
Body mass index <35 kg/m^2	Use of three or more anti-hypertensive drugs, which characterizes resistant hypertension
Glycated hemoglobin (HbA1c) between 7.0 and 10.5 %	Smoking within the previous 6 months
	Pregnancy or breastfeeding
	Creatinine clearance <45 mL/min (MDRD)
	Serum alanine aminotransferase or aspartate aminotransferase level of more than three times the upper limit of the normal range
	Subjects with ischemic heart disease, cerebrovascular disease, other atherosclerotic disease, cancer, or heart failure in functional classes II, III and IV
	Treadmill stress test with typical chest pain or with ST segment depression ≥1 mm, or with a horizontal or descending trace on the electrocardiogram for a duration of 0.08 s after the J point
	Presence of coronary heart disease diagnosed by treadmill stress test, myocardial scintigraphy or coronary angiography
	Inability to give informed consent

(100 mg/day b.i.d.) added-on to metformin (500–2550 mg/day according to glycemic control) and Group 2 will receive glibenclamide (5–20 mg/day according to glycemic control) added-on to metformin (500–2550 mg/day according to glycemic control). Blood samples will be collected after 12-h overnight fasting at screening visit and then after 12 weeks of treatment with vildagliptin (Group 1) or glibenclamide (Group 2). Renin-angiotensin system blockers will be prescribed to all subjects at the screening visit and all other antihypertensive drugs will be maintained. Subjects will have three return consultations. The first will be to randomize patients to Group 1 or 2 and they will be evaluated after four and 12 weeks. Compliance will be monitored by pill counts at the second and last visits. The glycemic control will be evaluated by glycated hemoglobin and fasting glucose. Adverse events will be evaluated on the basis of spontaneous reports and interviews by the investigator. Considering side effects and safety, nausea, upper abdominal pain and flatulence are expected, although uncommon, while taking vildagliptin. The most frequent side effect of glibenclamide is hypoglycemic events. Changes in blood count, renal function, and liver function are not expected with either treatment.

Table 2 shows a summary of key practical aspects of the study with all follow-up visits and requested exams.

Anthropometric measurements

Weight and height will be measured using metric weighing scales and a measuring tape and the body mass index (BMI) will be obtained using the formula: BMI = weight (kg)/(height in meters)^2. The waist circumference will be determined in centimeters using a measuring tape. This measurement will be carried out midway between the anterior superior iliac crest and the last rib at the end of expiration.

Measurement of blood pressure

BP will be measured in the office using a digital sphygmomanometer according to the VI Brazilian Guidelines on Hypertension Treatment [51]. Systolic (SBP) and diastolic blood pressure (DBP) will be recorded. Hypertension will be defined as a SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg or current use of anti-hypertensive drugs.

Biochemical tests

Blood samples will be drawn after 12 h of fasting to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), triglycerides (TG), glycemia, serum creatinine, alanine aminotransferase, glycated hemoglobin and ultrasensitive C-reactive protein (CRP). Turbidimetry (BioSystems) will be used to measure the CRP and the possibility of patients having had acute infectious or inflammatory processes within recent weeks will be excluded. Microalbuminuria will also be evaluated. To evaluate microalbuminuria, the urinary albumin-to-creatinine ratio (UACR) will be obtained from urine samples collected in the morning. Urine creatinine will be calculated using a colorimetric method, and albuminuria

Table 2 Key practical aspects of the study with all the clinical visits and the requested exams

Visits	Screening	Visit 1	Visit 2	Visit 3
Weeks	−1	1	4	12
Informed consent	X			
Inclusion and exclusion criteria	X	X		
Medical history	X			
Medical evaluation/physical examination	X	X	X	X
Randomization		X		
Pregnancy test	X			
Creatinine	X			X
Fasting glucose	X		X	X
Ultrasensitive C-reactive protein	X			X
Total cholesterol	X			X
High-density lipoprotein cholesterol	X			X
Triglycerides	X			X
Glycated hemoglobin	X			X
Alanine aminotransferase	X			X
Microalbuminuria	X			X
Ambulatory blood pressure monitoring		X		X
Treadmill test	X			
Determination of the central aortic pressure and vascular stiffness markers		X		X
Endothelium function		X		X

will be determined using the nephelometric method. The glomerular filtration ratio (GFR) will be estimated using the Modification of Diet in Renal Disease (MDRD) formula: GFR_{MDRD} (mL/min/1.73 m^2) = 186 (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) × (1.212 if black) [52].

Diabetic subjects will be identified by history of diabetes with dietetic treatment for diabetes or the use of hypoglycemic drugs. Subjects will be considered diabetics after two fasting glucose test results greater than 125 mg/dL according to the National Diabetes Data Group [53]. Serum cholesterol will be evaluated according to the Brazilian Guidelines for Dyslipidemias [54]. Low-density lipoprotein cholesterol (LDLc) will be calculated using the Friedewald formula for triglycerides levels below 400 mg/dL (LDLc = TC − HDLc − TG/5) [55].

Evaluation of coronary artery disease

At the first (screening) visit, all selected subjects will be evaluated for CAD using the treadmill stress test. Subjects with abnormal stress test results, including typical chest pain, with ST segment depression ≥1 mm, or with a horizontal or descending trace on the electrocardiogram for a duration of 0.08 s after the J point, will be excluded. When necessary, individuals will be submitted to other tests to evaluate CAD, such as myocardial scintigraphy and coronary angiography.

Measurement of blood pressure including 24-h ambulatory blood pressure monitoring

After randomization and again after 12 weeks of treatment with vildagliptin (Group 1) or glibenclamide (Group 2), 24-h non-invasive ambulatory blood pressure monitoring (ABPM) will be performed on a workday with a portable compact digital BP recorder (Mobil-O-Graph® 24-hour PWA monitor).

Automatic BP measurements will be recorded at 20-min intervals for diurnal readings (7.00 a.m.–11.00 p.m.) and at 30-min intervals for nocturnal readings (11.00 p.m.–7.00 a.m.). Nighttime and daytime periods will be assessed based on information reported by the subjects. The sleep BP will be defined as the mean BP from the time the subjects go to bed until the time they get up. The daytime BP will be defined as the average BP during the rest of the day. The subjects will be divided into two groups according to the dip in SBP during the nighttime: participants will be considered as dippers, if the decrease in sleep SBP is ≥10 % and non-dippers if the decrease is <10 %. As part of the exam protocol, all participants will be requested to make a note of their daily activities, their meal times, times of sleeping and waking

up, as well as time of taking medications and the presence of symptoms.

Vascular tests

Vascular tests will be performed in the subjects of both groups at the first visit and after 12 weeks of treatment.

1. Determination of endothelial function

Peripheral arterial tonometry (Endo-PAT 2000; Itamar Medical, Caesarea, Israel) is a non-invasive peripheral test of endothelial function [32]. The PAT device is placed on the tip of each index finger and comprises a pneumatic plethysmograph that applies a uniform pressure to the surface of the distal finger, allowing measurement of pulse volume changes. The inflation pressure of this digital device is electronically set at 10 mmHg below DBP or 70 mmHg. The PAT signal is recorded at baseline and then after 5 min of arterial occlusion using an inflatable cuff, while the contralateral arm serves as a control. The blood pressure cuff is inflated to 60 mmHg higher than systolic pressure or at least 200 mmHg. Lack of residual pulsatility is monitored throughout the occlusion period. Post-occlusive hyperemia stimulates endothelium-dependent vasodilatation, causing an increase in digital pulse amplitude. Pulse amplitude is recorded electronically in both fingers and analyzed by an automated, computerized algorithm (Itamar Medical). The change from the baseline measurement is expressed as the RHI, which, in part, reflects vasodilator function of the digital microcirculation.

Subjects will be instructed to fast the night before testing and to refrain from ingesting alcohol or caffeine. The room temperature will be maintained between 21 and 24 °C at all times during the exam; restrictive clothing, watches, rings, or other jewelry on the hands that might interfere with blood flow will be removed. The test will be performed in the morning after the patient has been comfortably seated or has laid down in the study room for at least 15 min to reach a relaxed cardiovascular steady-state [31, 32]. The subjects of the study will be submitted to the endothelial function test at their first visit and after 12 weeks of treatment. Endothelial dysfunction will be defined as a RHI $\leq$ 1.68, according to a study performed in healthy asymptomatic control individuals without history of CVD and without major risk factors [56].

2. Determination of the central aortic pressure and vascular stiffness

Arterial stiffness, assessed using the non-invasive method of radial artery applanation tonometry, is predictive of vascular disease. A portion of the artery pressure wave travelling towards the extremities is reflected back by peripheral impedance points. In healthy individuals, the reflected wave returns to the aorta during diastole. However, when arteries become stiff, the transit time between the incident and reflected waves is reduced. Thus, the reflected wave returns to the aorta during systole of the same cardiac cycle thereby augmenting the central BP. This elevation of the central BP can be quantified using the AIx [57, 58]. The AIx is associated with cardiovascular risk, and predicts the presence or absence of CAD [59]. Higher values of the AIx indicate increased wave reflection from peripheral vessels or earlier return of the reflected wave as a result of increased pulse wave velocity, which is attributed to an increased arterial stiffness. In young healthy individuals, the systolic arterial pressure (aortic) is about 15–20 mmHg lower than the peripheral systolic pressure (brachial) [45, 60].

Outcomes and outcome adjudication

1. Change in the RHI from baseline after 12 weeks of vildagliptin vs. glibenclamide treatment.
2. Change in the central blood pressure from baseline to after 12 weeks of vildagliptin vs. glibenclamide treatment.

Statistical considerations

Sample size and power calculations

The site http://www.lee.dante.br-pesquisa was used to estimate the sample size, considering a 30 % change in the RHI between treatment groups as clinically relevant. Assuming a standard deviation of 0.3 would require a total of 21 subjects to detect a 30 % change in the RHI with a power of 80 % at a significant level of 0.05. However, considering a potential 20 % of drop-out or lost to follow-up rate, a total of 50 patients (25 for each group) will be considered.

Statistics

All analysis will be performed using SPSS Statistical Software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Continuous variables will be presented as mean $\pm$ SD and categorical variables as frequencies. Differences between the both groups at baseline will be evaluated by unpaired *t* test or the Mann–Whitney test for comparison of continuous variables. The Chi square test or Fisher's exact test will be employed to compare categorical variables. The change from baseline to 12-weeks follow-up in the both groups will be evaluated using the paired *t* test for continuous variables. Pearson's correlation will be used to assess the relationship between HbA1c and RHI and AIx, after confirmation of similarity between groups in respect to demographic data (age, gender, GFR, and comorbidities:

hypertension and dyslipidemia) and HbA1c targets after 12 weeks of considered treatment. Thus, the Pearson's correlation obtained will be potentially consequent to distinct therapeutic responses. For all analysis, a 2-sided $P < 0.05$ will be considered statistically significant.

Results/discussion

The study has started in December 2013 and patient recruitment is programed until October 2015. It is expected that vildagliptin will improve the endothelial function in patients with T2DM and hypertension more than glibenclamide treatment. Hyperglycemia causes endothelial dysfunction because it reduces the bioavailability of endothelium-derived nitric oxide (NO). Although the main action of GLP-1 is to increase glucose-stimulated insulin secretion from pancreatic beta cells, there is also evidence of physiological signaling from GLP-1 in endothelial and vascular smooth muscle cells. Thus, GLP-1 has vasodilator actions mediated by a specific GLP-1 receptor in the vascular endothelium and may improve endothelial function in animals and humans [15–17], independently of its effect on glycemic control.

Arterial stiffness is recognized as another cardiovascular risk marker [32]. Patients with both HT and DM exhibit higher arterial stiffness compared to those with either DM or HT alone [33]. Applanation tonometry estimates arterial compliance and the central blood pressure and is used to assess arterial stiffness [42, 43]. Evidence shows that the central blood pressure is more relevant to cardiovascular outcomes than the peripheral BP [44–46]. On the other hand, Endo-PAT, a non-invasive method, evaluates the vascular vasodilator propriety and, consequently, endothelial function. Both hypoglycemic drugs, vildagliptin and glibenclamide, may improve glycemic control. However, only vildagliptin and other DPP-4 inhibitors have provided beneficial effects of the endothelium [22, 23].

Conclusions

This study will evaluate the effect of vildagliptin compared to glibenclamide, both added-on to metformin, on endothelial function and arterial stiffness in type 2 diabetic patients with hypertension. The improvement of endothelial function will demonstrate that DPP-4 inhibitors could improve cardiovascular outcome, especially in high cardiovascular risk patients.

Abbreviations

ABPM: ambulatory blood pressure monitoring; ADMA: asymmetric dimethylarginin; AIx: *augmentation index*; BMI: body mass index; BP: blood pressure; CAD: coronary artery disease; CRP: C-reactive protein; CVD: cardiovascular disease; DBP: diastolic blood pressure; T2DM: type 2 diabetes mellitus; DPP-4: dipeptidyl peptidase-4; FMD: flow-mediated vasodilatation; GIP: glucose-dependent insulinotropic polypeptide; GFR: glomerular filtration ratio; GLP-1: glucagon-like peptide 1; HDLc: High-density lipoprotein cholesterol; HT: hypertension; LDLc: Low-density lipoprotein cholesterol; MDRD: Modification of Diet in Renal Disease; PAI-1: plasminogen activator inhibitor 1; PAT: peripheral arterial tonometry; PWV: pulse wave velocity; RHI: reactive hyperemia index; SBP: systolic blood pressure; SD: standard deviation; TC: total cholesterol; TG: triglycerides; UACR: urinary albumin-to-creatinine ratio.

Authors' contributions

LNCM and JFVM conceived the study; LNCM and JFVM had overall responsibility for the study and initial drafting of the text; LNCM, LTGJr, DDM, CBC, JPC and JFVM were responsible for the day-to-day operationalization and management of the study; MAN was involved in completing the statistical analyses. All authors participated in the trial design and methodological considerations, contributed to the draft of this manuscript for intellectual content and approved its final version. All authors read and approved the final manuscript.

Author details

[1] Hospital de Base/Centro Integrado de Pesquisa da Fundação Faculdade Regional de Medicina de São José do Rio Preto (FUNFARME), São José do Rio Preto, Brazil. [2] Internal Medicine Department and Hypertension Clinic, State Medical School in São José do Rio Preto (FAMERP), Ave Brig. Faria Lima 5416, São José do Rio Preto, SP 15090-000, Brazil.

Acknowledgements

This study is sponsored by Novartis. However, Novartis did not have any influence in the study design, methods, data management or analysis. The authors would like to thank the patients and staff who are participating in this clinical trial. We would also like to thank all the authors that contributed equally to the literature search, data interpretation, figure creation, and writing of the manuscript. We thank the reviewer David Hewitt for correcting both the spelling and grammar of the English text.

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests. Novartis did not have any influence in the study design, methods, data management or analysis.

Ethical approval

The project was approved by the Ethics Committee of the Medical School in São José do Rio Preto (CAAE no 11665513.7.0000.5415, no 211.243/2013), which is accredited by the Office of Protection of Human Research as an Institutional Review Board. All participants will sign an informed consent form to participate.

Grants

Novartis funded the study.

Received: 15 April 2015 Accepted: 7 August 2015
Published online: 26 August 2015

References

1. Ministry of Health. DATASUS. Information about health: cardiovascular morbidity and mortality. http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sih/cnv/niuf.def. Accessed 02 Dec 2014.
2. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Heart Disease and Stroke Statistics—2014 update: a report from the American Heart Association. Circulation. 2013;127(1):e6–245.
3. Cipullo JP, Martin JF, Ciorlia LA, Godoy MR, Cação JC, Loureiro AA, et al. Hypertension prevalence and risk factors in a Brazilian urban population. Arq Bras Cardiol. 2010;94:519–26.
4. Bosi PL, Carvalho AM, Contrera D, Casale G, Pereira MA, Gronner MF, et al. Prevalence of diabetes and impaired glucose tolerance in the urban population of 30 to 79 years of the city of São Carlos, São Paulo. Arq Bras Endocrinol Metab. 2009;53:726–32.
5. Moraes SA, Freitas IC, Gimeno SG, Mondini L. Diabetes mellitus prevalence and associated factors in adults in Ribeirão Preto, São Paulo, Brazil, 2006: OBEDIARP Project. Cad Saude Publica. 2010;26:929–41.

6. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;94:311–21.
7. Collaboration Emerging Risk Factors, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet. 2010;375:2215–22.
8. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287:1420–6.
9. Penny WF, Ben-Yehuda O, Kuroe K, Long J, Bond A, Bhargava V, et al. Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol. 2001;37:766–74.
10. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, David H, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101:948–54.
11. Perrone-Filardi P, Cuocolo A, Brevetti G, Silvestro A, Storto G, Dellegrottaglie S, et al. Relation of brachial artery flow-mediated vasodilatation to significant coronary arterial disease in patients with peripheral arterial disease. Am J Cardiol. 2005;96:1337–41.
12. Mäkimattila S, Virkamäki A, Groop PH, Cockcroft J, Utriainen T, Fagerudd J, Yki-Järvinen H. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. Circulation. 1996;94:1276–82.
13. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368:1696–705.
14. Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, Roman RJ. Antihypertensive effect of glucagons-like peptide 1 in Dahl salt-sensitive rats. J Hypertens. 2003;21:1125–35.
15. Nyström T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahrén B, Sjöholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary arterial disease. Am J Physiol Endocrinol Metab. 2004;287:E1209–15.
16. Ceriello A, Esposito K, Testa R, Bonfigli AR, Marra M, Giugliano D. The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an "endothelial resistance" to the glucagon-like peptide 1 in diabetes. Diabetes Care. 2011;34:697–702.
17. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Druker DJ, Husain M. Cardioprotective and vasodilatadory actions of glucagons-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation. 2008;117:2340–50.
18. Gurkan E, Tarkun I, Sahin T, Cetinarslan B, Canturk Z. Evaluation of exenatide versus insulin glargine for the impact on the endothelial functions and cardiovascular risk markers. Diabetes Res Clin Pract. 2014;106:567–75.
19. Forst T, Michelson G, Ratter F, Weber MM, Anders S, Mitry M, et al. Addition of liraglutide in patients with Type 2 diabetes well controlled on metformin monotherapy improves several markers of vascular function. Diabet Med. 2012;29:1115–8.
20. Gejl M, Sondergaard HM, Stecher C, Bibby BM, Moller N, Botker HE, et al. Exenatide alters myocardial glucose transport uptake and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. J Clin Endocrinol Metab. 2012;97:E1165–9.
21. Subaran SC, Sauder MA, Chai W, Jahn LA, Fowler DE, Aylor KW, et al. GLP-1 at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. Clin Sci (Lond). 2014;127:163–70.
22. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal derived factor-1 alpha. Diabetes Care. 2010;33:1607–9.
23. Van Poppel PC, Netea MG, Smits P, Tack CJ. Vildagliptin improves endothelium-dependent vasodilatation in type 2 diabetes. Diabetes Care. 2011;34:2072–7.
24. Faber R, Zander M, Pena A, Michelsen MM, Mygind ND, Prescott E. Effect of glucagon-like peptide-1 analogue liraglutide on coronary microvascular function in patients with type 2 diabetes—a randomized, single-blinded, cross-over pilot study. Cardiovasc Diabetol. 2015;14:41.
25. Ayaori M, Iwakami N, Uto-Kondo H, Sato H, Sasaki M, Komatsu T, et al. Dipeptidyl peptidase-4 inhibitors attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2 diabetic patients. J Am Heart Assoc. 2013;2:e003277.
26. Scirica BM, Bhatt DL, Braunwald E, Steg G, Davidson J, Hirshberg B, et al. for the SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med. 2013;369:1317–1326.
27. White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, et al. for the EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med. 2013;369:1327–1335.
28. Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, et al. TECOS Study Group. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2015;373(3):232–42.
29. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J. 2010;31:1142–8.
30. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J. 2003;146:168–74.
31. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, et al. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. J Am Coll Cardiol. 2003;41:1761–8.
32. Bonetti PO, Pumper GM, Higano ST, Holmes DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol. 2004;44:2137–41.
33. Kuvin JT, Mammen A, Mooney P, Alsheikh-Ali AA, Karas Rh. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vasc Med. 2007;12:13–6.
34. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation. 2004;109:184–9.
35. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension. 2007;49:1202–6.
36. Tedesco MA, Natale F, Di Salvo G, Caputo S, Capasso M, Calabró R. Effects of coexisting hypertension and type II diabetes mellitus on arterial stiffness. J Hum Hypertens. 2004;18:469–73.
37. Gedikli O, Kiris A, Yilmaz H, Ozturk S, Baykan M, Durmus I, et al. The relationship between endothelial damage and aortic augmentation index. Clin Exp Hypertens. 2010;32:29–34.
38. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113:657–63.
39. Mulè G, Cottone S, Cusimano P, Riccobene R, Palermo A, Geraci C, et al. The association of microalbuminuria with aortic stiffness is independent of C-reactive protein in essential hypertension. Am J Hypertens. 2009;22:1041–7.
40. Hermans MM, Henry R, Dekker JM, Kooman JP, Kostense PJ, Nijpels G, et al. Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness: the Hoorn study. J Am Soc Nephrol. 2007;18:1942–52.
41. Takenaka T, Mimura T, Kanno Y, Suzuki H. Qualification of arterial stiffness as a risk factor to the progression of chronic kidney disease. Am J Nephrol. 2005;25:417–24.
42. Nürnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schäfers RF. Augmentation index is associated with cardiovascular risk. J Hypertens. 2002;20:2407–14.
43. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation. 2006;113:664–70.
44. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55:1318–27.
45. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. Hypertension. 2001;38:932–7.

46. DeLoach SS, Townsend RR. Vascular stiffness: its measurements and significance for epidemiologic and outcome studies. Clin J Am Soc Nephrol. 2008;3:184–92.
47. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation. 2006;113:1213–25.
48. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. Hypertension. 2007;50:197–203.
49. Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guvonvarc'h PM, et al. Central pulse pressure and mortality in end-stage renal disease. Hypertension. 2002;39:735–8.
50. Cosenso-Martin LN, Giollo-Junior LT, Vilela-Martin JF. DPP-4 inhibitor reduces central blood pressure in a diabetic and hypertensive patient. Medicine (Baltimore). 2015;94:e1068.
51. Brazilian Society of Cardiology, Brazilian Society of Hypertension, Brazilian Society of Nephrology. VI Brazilian guidelines on hypertension. Arq Bras Cardiol. 2010;95(1 Supl 1):1–51.
52. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461–70.
53. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care. 2003;26:3160–7.
54. Atherosclerosis Department of Brazilian Society of Cardiology. IV Brazilian guidelines on dyslipidemia and atherosclerosis prevention. Arq Bras Cardiol. 2007;88(Suppl I):1–19.
55. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
56. Gargiulo P, Marciano C, Savarese G, D'Amore C, Paolillo S, Esposito G, et al. Endothelial dysfunction in type 2 diabetic patients with normal coronary arteries: a digital reactive hyperemia study. Int J Cardiol. 2013;165:67–71.
57. Munir S, Guilcher A, Kamalesh T, Clapp B, Redwood S, Marber M, Chowienczyk P. Peripheral augmentation index defines the relationship between central and peripheral pulse pressure. Hypertension. 2008;51:112–8.
58. Giollo Junior LT, Gomes MAM, Vilela-Martin JF. The evaluation of antihypertensive response with applanation tonometry. Rev Bras Hipertens. 2010;17:189–90.
59. Patvardhan E, Heffernan KS, Ruan J, Hession M, Warner P, Karas RH, Kuvin JT. Augmentation index derived from peripheral arterial tonometry correlates with cardiovascular risk factors. Cardiol Res Pract. 2011;2011:253758.
60. Bortolotto LA. Central pressure: how to understand in clinical practice? Rev Bras Hipertens. 2009;16:46–7.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central

2.1.3 ARTIGO 3

2.1.3 ARTIGO 3

The Effect of Vildagliptin on Endothelial Function, Arterial Stiffness and Blood Pressure in Patients with Type 2 Diabetes and Hypertension

Short running title: Vildagliptin and Endothelial Function

Luciana Neves Cosenso-Martin, Luiz Tadeu Giollo-Júnior, Marcelo Arruda Nakazone, Marco Motta, Maurício de Nassau Machado, José Fernando Vilela-Martin

Internal Medicine Department and Hypertension Clinic, State Medical School at São José do Rio Preto / São Paulo / Brazil (FAMERP) and Hospital de Base at São José do Rio Preto/SP/Brazil

Correspondence to:
José Fernando Vilela-Martin, MD PhD FAHA
Ave Brig. Faria Lima 5416 - Postal Code: 15090-000
São José do Rio Preto / SP / Brazil, Phone (fax): +55 17 32015727
e-mail: vilelamartin@uol.com.br

Abstract

OBJECTIVE

Several trials have shown that dipeptidyl peptidase-4 (DPP-4) inhibitors, used to treat type 2 diabetes (T2DM), improve endothelial function. The current study investigated the effects of vildagliptin, a DPP-4 inhibitor, compared to glibenclamide on endothelial function and arterial stiffness (AS) in patients with T2DM and hypertension.

RESEARCH DESIGN AND METHODS

This trial was a prospective randomized, open label, controlled by drug. Patients aged over 35 years with T2DM and hypertension, without cardiovascular disease, were randomly allocated to treatment with vildagliptin (n=25) or glibenclamide (n=25). Both groups used metformin. Endothelial function was evaluated by peripheral artery tonometry (Endo-PAT 2000), measuring the reactive hyperemia index (RHI) and AS. The primary outcome was change in the RHI after 12 weeks of vildagliptin vs. glibenclamide treatment. A 24-h non-invasive ambulatory blood pressure monitoring was performed before and after 12 weeks of treatment. AS was evaluated by augmentation index (AIx75), pulse wave velocity (PWV) and central systolic blood pressure (cSBP) parameters.

RESULTS

There were no changes in the RHI in the vildagliptin group (before 2.348 ± 0.5868; after 2.2408 ± 0.6019, P=0.742) or in the glibenclamide group (2.3636 ± 0.5163 and 2.3375 ± 0.4996, P=0.950), and no differences between groups (P=0.5479). There was also no difference between vildagliptin and glibenclamide treatment in AIx75 (P=0.696), in cSBP (P=0.274) and in PWV (P=0.324).

CONCLUSIONS

Vildagliptin, in patients with T2DM and hypertension, does not change endothelial function and AS during 12 weeks. Thus, this drug has a neutral effect on vascular function.

Key-words: arterial stiffness, diabetes, hypertension, endothelial function, pulse wave velocity, vildagliptin

Introduction

Cardiovascular disease (CVD) is the main cause of deaths in developing and developed countries. Hypertension (HT) and type 2 diabetes mellitus (T2DM) are among the main causes of CVD (1). T2DM is associated with a twofold higher risk for CVD (2). Endothelial dysfunction is an independent predictor for future CVD in patients with T2DM (3) and is considered an early marker of vascular complications (4). Thus, the T2DM management includes reduce the incidence of CVD.

Today, some groups of drugs that act on the incretin system, such as glucagon like peptide-1 (GLP-1) analogues/agonists and dipeptidyl peptidase-4 enzyme (DPP-4) inhibitors, are used to treat T2DM and may be responsible for beneficial effects on endothelial function (5). Those effects might be through a specific GLP-1 receptor in the vascular endothelium (6) and mediated by the metabolites of GLP-1 (7). One study on exenatide, a GLP-1 agonist, showed a significant increase in flow-mediated vasodilatation (FMD) (8).

Vildagliptin, a DPP-4 inhibitor, improved endothelium dependent vasodilatation with an invasive method in T2DM patients (9). In contrast, other studies did not demonstrate same effects (10,11).

Recently, three large multicenter, randomized trials testing saxagliptin (12), alogliptin (13), and sitagliptin (14) did not find any reduction in cardiovascular events. However, these drugs are considered safe for patients with CVD.

Several variables have been used to assess impaired endothelial function and arterial stiffness (15). Among them, FMD and peripheral arterial tonometry (PAT) are very useful. PAT is a simple, non-invasive, and reproducible technique used to assess endothelial function and, in patients with CAD, has been shown to strongly correlate with the parameters of endothelial dysfunction (16-17). It measures volume changes in the fingertip before and after blood flow occlusion and automatically calculates the reactive hyperemia index (RHI), providing an index for endothelial function. Furthermore, it evaluates arterial stiffness (AS) calculating augmentation index (AIx).

Arterial stiffness is another parameter used to investigate endothelial dysfunction. Patients with both HT and T2DM exhibit increased arterial stiffness compared to those with either T2DM or HT alone (18). The AIx is a marker of wave reflection derived from aortic pressure wave analysis, with increased AIx being correlated to increased

stiffness and contributing to cardiovascular risk (19-20). Evidence shows that the central blood pressure (BP) is more relevant to cardiovascular outcomes than the BP in the brachial artery (21). As the AIx is influenced by heart rate, a index normalized for a heart rate at 75 bpm (AIx75) was used in accordance with Wilkinson et al (22). More recently, a study demonstrated improvement in central BP and AIx75 following the use of vildagliptin in a patient with T2DM and HT (23).

Thus**,** the purpose of this study was to evaluate the effect of vildagliptin compared to glibenclamide both added-on to metformin on endothelial function and arterial stiffness in patients with T2DM and hypertension.

Research Design and Methods

Study Design

This trial was a randomized, open label, controlled by drug as described previously (24). This study was registered on **clinicaltrials.gov identifier: NCT02145611**. It was designed to assess the effect of vildagliptin 50 mg twice a daily (bid) on endothelial function in patients with T2DM and hypertension compared to glibenclamide (5-20 mg/day depending on glycemic control).

Study Population

Between July 2013 and February 2016, 112 patients from the diabetes and hypertension outpatient clinics at the Hospital de Base, FAMERP, Brazil were invited to participate in this study. The Research Ethics Committee of the institution approved the study protocol according to national and international guidelines. All patients gave their informed consent. The inclusion criteria were: history of T2DM and mild hypertension no longer than 15 years, age ≥ 35 years, HbA1c between 7.0 (53 mmol/L) and 10.5% (91 mmol/L), body mass index (BMI) < 35Kg/m^2. The exclusion criteria were: use of insulin, pioglitazone, GLP-1 receptor agonist, DPP-4 inhibitor or acarbose, intolerance to metformin, smoking, resistant hypertension, cardiovascular disease, cerebrovascular disease, creatinine clearance < 45 mL/min, altered treadmill stress test.

Randomization and Study Intervention

A computer-validated software (Random allocator) was used for random allocation.

Twenty-five individuals with T2DM and hypertension were evaluated in the vildagliptin plus metformin group compared to 25 diabetic and hypertensive subjects in the glibenclamide plus metformin group before and after 12 weeks of treatment.

Observation Variables and Schedule

The study period was 12 weeks after randomization. Clinical outcome, adherence, and adverse events were confirmed, and clinical and biochemical data were collected at 0 and 12 weeks after randomization.

Study Outcomes

The primary study outcome was change in the endothelial function evaluated by RHI from baseline after 12 weeks of vildagliptin vs. glibenclamide treatment.

The secondary outcomes were change in the AS and blood pressure from baseline after 12 weeks of vildagliptin vs. glibenclamide treatment. AS was evaluated by AIx75, central pulse pressure (PP), central blood pressure and pulse wave velocity (PWV).

Measurement of Endothelial Function

Peripheral arterial tonometry (Endo-PAT 2000; Itamar Medical, *Caesarea*, Israel) is a non-invasive peripheral test of endothelial function (17). This test was performed at baseline and after 12 weeks of treatment as described previously (24). This device also evaluated AIx75.

24-h blood ambulatory blood pressure monitoring (ABPM)

The 24-h blood pressure monitoring was performed by a portable compact digital BP recorder (Mobil-O-Graph® 24-hour PWA monitor). This device evaluates the central aortic pressure waveform and can be used to determine central systolic and diastolic BP, central PP, AIx75, PWV during 24 hours. Central PP and AIx75 are markers of arterial stiffness showing good correlation with cardiovascular morbidity and mortality (19,20). PWV is a direct measure of arterial stiffness of large arteries.

Biochemical Tests

Blood samples were drawn after 12 hours of fasting to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), triglycerides (TG), glycemia, serum creatinine, alanine aminotransferase, glycated hemoglobin (HbA1c). The glomerular filtration ratio (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) formula: GFR_{MDRD} (mL/min/1.73 m^2) = 186 (serum creatinine) $^{-1.154}$ × (age) $^{-0.203}$ × (0.742 if female) × (1.212 if black) (25).

Statistical Analysis

All analysis was performed using SPSS Statistical Software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Continuous variables were presented as mean ± SD and categorical variables as frequencies. Differences between the both groups at baseline were evaluated by unpaired **t**-test or the Mann-Whitney test for comparison of continuous variables. The chi-square test or Fisher's exact test was employed to compare categorical variables. The change from baseline to 12-weeks follow-up in the both groups was evaluated using the paired **t** test for continuous variables. Pearson's correlation was performed to assess the relationship between HbA1c and RHI and AIx, after confirmation of similarity between groups in respect to demographic data (age, gender, GFR, and comorbidities: hypertension and dyslipidemia) and HbA1c targets after 12 weeks of considered treatment. All statistical were two-sided with 5% significance level.

Results

The majority of screen failure was due to HbA1c levels (41 patients), followed by altered treadmill stress test (9 patients), BMI above 35 (3 patients) and others. Fifty patients were enrolled in the study and 48 (24 for each group) completed the study. One patient in the vildagliptin was withdrawn because of a worsening of renal function not related to the drug. Another patient in the glibenclamide group was lost to follow-up. The baseline demographic and clinical characteristics of all subjects and the effects of each treatment are shown in Table 1. There were no differences between the 2 groups in age, duration of T2DM and HT, BMI, HbA1c level, concomitant use of medications, except for aspirin, more common in the glibenclamide group (P = 0.037). There was no

statistically significant difference in heart rate, BP, BMI, eGFR within and between groups after treatment.

Although the vildagliptin group demonstrated reduction of total cholesterol, LDL cholesterol and triglycerides ($P < 0.05$), the difference between groups was not significant. There was no difference in metformin use, comparing the groups (1789.583 ± 603.9686 mg/day and 1750 ± 726.5164 mg/day, for vildagliptin and glibencalmide groups, respectively, $P = 0.983$). The mean glibenclamide use was 12 ± 4.472 mg/day in the group 2.

There were no changes in RHI in the vildagliptin group (before 2.348 ± 0.5868; after 2.2408 ± 0.6019, $P = 0.742$) or in the glibenclamide group (before 2.3636 ± 0.5163; after 2.3375 ± 0.4996, $P = 0.950$) (Figure 1). There was also no difference in RHI between groups ($P = 0.5479$). In addition, there was no change in AIx75 PAT within groups ($P = 0.696$ and 0.819 for vildagliptin and glibenclamide, respectively).

Both groups presented similar decreases in HbA1c ($P = 0.1814$ and $P = 0.1709$ in the vildagliptin and glibenclamide groups). However, fasting glucose decreased significantly in both groups ($P = 0.0185$ and $P = 0.0170$ in the vildagliptin and glibenclamide groups, respectively), but without difference between groups ($P = 0.5676$), as shown in the Table 1.

In relation to ABPM, the patients in two groups significantly decreased 24-hour: systolic blood pressure (SBP), diastolic BP (DBP), mean blood pressure (MBP) and periferic pressure pulse (PPP) compared to baseline values ($P = 0.032$, 0.027, 0.022 and 0.039 in the vildagliptin group; $P = 0.002$, 0.002, 0.002 and 0.016 in glibenclamide group) (Table 2). However there was no difference between the groups $P = 0.658$, 0.928, 0.865 and 0.609, respectively). Additionally, a reduction in 24-hour central SBP (cSBP) and 24-hour PWV occurred in both groups, but significantly only in the glibenclamide group ($P = 0.003$ for both parameters), without difference comparing with vildagliptin ($P = 0.274$ and 0.324 for cSBP and PWV, respectively). There was no change in 24-hour AIx75 and in central PP in both groups ($P = 0.988$ and 0.329 in the vildagliptin group; $P = 0.376$ and 0.101 in the glibenclamide group).

Pearson's correlation demonstrated that there was not correlation between glycemic control, BP and arterial stiffness parameters and RHI in both groups. In contrast, age, central PP and office BP were correlated with PWV after 12 weeks in both groups ($P <$

0.001, P = 0.008 and 0.008 for vildagliptin; P <0.001, 0.001 and <0.001 for glibenclamide). AIx75 PAT was correlated with PWV only in the glibenclamide group (P = 0.007), whereas cSBP and SBP were correlated with PWV only in the vildagliptin (P = 0.006 and 0.002).

No serious adverse event was reported. Although hypoglycemic events were reported in two patients in the glibenclamide group, this was not different (P = 0.149). In the vildagliptin group, one patient finished his treatment earlier (7 weeks) due to hyperglicemic event, and insulin was started, but it was not ketoacidosis. One patient in the vildaglipitn was withdrawn because a worsening of renal function not related to the drug.

Conclusions

This was a randomized, open label, prospective study that compared the effect of vildagliptin vs. glibenclamide added-on to metformin on endothelial function in hypertensive and diabetic patients. Similar degrees of improvement in glycemic control were achieved in the vildagliptin and glibenclamide groups. According to a previous study with pooled data on vildagliptin monotherapy (26), this trial also demonstrated reduction of total cholesterol, LDL cholesterol and triglycerides (P < 0.05) in the vildagliptin group, but difference for glibenclamide group was not significant. Differently of present study, the sample size was large (> 2000 naïve patients), for 24 weeks of treatment and no data of confounding blood pressure and lipid medication were collected.

However, vildagliptin did not alter endothelial function assessed by Endo-PAT 2000, similarly to a trial with liraglutide, a GLP-1 analogue, in RHI (10). The effect of vildagliptin on endothelial function has been controversially reported. A recent research observed vascular function improvement by measuring forearm blood flow during Ach infusion (9). Although it might be attributable to a difference in methodology, Endo-PAT 2000, used in the present study, also evaluates resistance artery (not invasive). Differently from them (9), endothelial function was performed before and after treatments, while they measured vascular function only after the treatments. Additionally, sitaglipin improved FMD in diabetic subjects (27). However, the study

was performed in a single arm, without a control group. Other studies with sitagliptin (28) and linagliptin (29) did not alter endothelial function, while sitagliptin and alogliptin attenuated endothelial function by FMD (11).

Our study did not demonstrate effect of DPP-4 inhibitor on arterial stiffness, contrasting with recent studies with linagliptin, which prevented western diet-induced aortic stiffening in female mice (30) and with sitagliptin and vildagliptin, which reduced AIx75, cSBP after 8 weeks of treatment in 51 T2DM (31). Although the study was similar to the present study, some differences can be observed. In our study, all patients had hypertension, CVD was excluded according to image methods and not only by history and renin-angiotensin system blockers were prescribed to all subjects. Thus, those factors could explain the lack AS improvement in our subjects**.** In contrast, our results are comparable to two other researchers, which demonstrated neither sitagliptin nor glibenclamide (32) or vildagliptin added to metformin had effect on AS in subjects with T2DM (33). Furthermore, Gordin D et al demonstrated that AS in T2DM subjects is similar to controls during fasting. Thus, they concluded that rigorous control of BP with renin-angiotensin system blockers and cholesterol with statins could decrease the development of arterial stiffness in T2DM (34).

At present study, there was improvement of 24h-SBP, 24-DBP and 24-h PPP in both groups, similarly to previously described (31). Although this effect is mainly due the hypertensive treatment and no related to the diabetic treatment, Jackson et al demonstrated that the effects of DPP-4 inhibitors on BP are context dependent (35).

Although recent researchers demonstrated anti-atherosclerotic effects of DPP-4 inhibitors (36-39), our results have not demonstrated beneficial effect of vildagliptin on vascular function. A recent experimental study demonstrated that sitagliptin acts against oxidative stress in rats not receiving renin-angiotensin system blockers (40). As all subjects in our study used renin-angiotensin system blockers, this might explain no changes of vildagliptin on vascular function.

The results of this trial are according with 3 large multicenter, randomized trials (12-14), which did not find any reduction in cardiovascular events. Although this is a small sample size and only 12 weeks of treatment, this is the first study that included diabetic and hypertensive patients without CVD, with control of variables that influence in endothelial function, such as smoking, insulin and renin-angiotensin system blockers.

Finally, as there was not effect of vildagliptin on endothelial function and arterial stiffness, others randomized trials are necessary to confirm the beneficial effect of the DPP-4 inhibitors on vascular function.

Acknowledgements:

The authors would like to thank the patients and staff who are participating in this clinical trial. We would also like to thank all the authors that contributed equally to the literature search, data interpretation, figure creation, and writing of the manuscript. We thank the reviewer David Hewitt for correcting both the spelling and grammar of the English text and Lilian Castiglioni for the statistic analysis.

Author conflicts of interest/disclosure information

This study was sponsored by Novartis. However, Novartis did not have any influence in the study design, methods, data management or analysis.

Funding information: Novartis

References

1. Heart Disease and Stroke Statistics - 2014 Update: A Report From the American Heart Association. Circulation 2013;127: e6-e245

2. Emerging Risk Factors Collaboration. Sarwar N, Gao P, Seshasai SR, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 2010;375:2215-2222

3. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002;287:1420-1426

4. Penny WF, Ben-Yehuda O, Kuroe K, et al. Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol 2001;37:766-774

5. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 2006;368:1696-1705

6. Ceriello A, Esposito K, Testa R, et al. The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an "endothelial resistance" to the glucagon-like peptide 1 in diabetes. Diabetes Care 2011;34:697-702

7. Ban K, Noyan-Ashraf MH, Hoefer J, et al. Cardioprotective and vasodilatadory actions of glucagons-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and –independent pathways. Circulation 2008;117:2340-2350

8. Gurkan E, Tarkun I, Sahin T, et al. Evaluation of exenatide versus insulin glargine for the impact on the endothelial functions and cardiovascular risk markers. Diabetes Res and Clin Pract 2014;106:567-575

9. Van Poppel PC, Netea MG, Smits P, et al. Vildagliptin improves endothelium dependent vasodilatation in type 2 diabetes. Diabetes Care 2011;34:2072-2077.

10. Faber R, Zander M, Pena A, et al. Effect of glucagon-like peptide-1 analogue liraglutide on coronary microvascular function in patients with type 2 diabetes – a randomized, single-blinded, cross-over pilot study. Cardiovascular Diabetol 2015;14:41

11. Ayaori M, Iwakami N, Uto-Kondo H, et al. Dipeptidyl peptidase-4 inhibitors

attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2 diabetic patients. J Am Heart Assoc 2013; 2:e003277

12. Scirica BM, Bhatt DL, Braunwald E, et al, for the SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. N Engl J Med 2013;369:1317-1326

13. White WB, Cannon CP, Heller SR, et al, for the EXAMINE Investigators. Alogliptin after Acute Coronary Syndrome in Patients with Type 2 Diabetes. N Engl J Med 2013;369:1327-1335

14. Green JB, Bethel MA, Armstrong PW, et al. TECOS Study Group. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. N Engl J Med 2015; 373(3):232-242

15. Balduino Mendes AB, Giollo Jr LT, de Andrade DO, et al. How to investigate the vascular changes in Resistant Hypertension. Curr Hypertens Rev 2016;12:139-147.

16. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J. 2003;146:168-74.

17. Bonetti PO, Pumper GM, Higano ST, et al. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol 2004;44:2137-2141

18. Tedesco MA, Natale F, Di Salvo G, et al. Effects of coexisting hypertension and type II diabetes mellitus on arterial stiffness. J Hum Hypertens 2004;18:469-473

19. Willum-Hansen T, Staessen JA, Torp-Pedersen C, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 2006;113:664-670

20. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:1318-1327

21. Williams B, Lacy PS, Thom SM, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 2006;113:1213-1225.

22. Wilkinson IB, MacCallum H, Flint L, et al. The influence of heart rate on augmentation index and central arterial pressure in human. J Physiol 2000;525:263-270

23. Cosenso-Martin LN, Giollo-Junior LT, Vilela-Martin JF. DPP-4 inhibitor reduces central blood pressure in a diabetic and hypertensive patient. Medicine (Baltimore) 2015;94:e1068

24. Cosenso-Martin LN, Giollo-Junior LT, Martinelli DD, et al. Twelve-week randomized study to compare the effect if vildagliptin vs. glibenclamide both added-on to metformin on endothelium function in patients with type 2 diabetes and hypertension. Diabetol Metab Syndr 2015;7:70

25. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. Ann Intern Med. 1999;130:461-470

26. Evans M, Schweizer A, Foley JE. Blood pressure and fasting lipid changes after 24 weeks' treatment with vildagliptin: a pooled analysis in > 2,000 previously drug-naïve patients with type 2 diabetes mellitus. Vasc Health Risk Man. 2016;12:337-340

27. Kubota Y, Miyamoto M, Takagi G, et al. The dipetidyl peptidase-4 inhibitor sitagliptin improves vascular endothelial function in type 2 diabetes. JKMS 2012;27:1364-1370

28. Maruhashi T, Higashi Y, Kihara Y, et al. Long-term effect of sitagliptin on endothelial function in type 2 diabetes: a sub-analysis of the PROLOGUE study. Cardiovasc Diabetol 2016;15:134

29. Baltzis D, Dushay JR, Loader J, et al. Effect of linagliptin on vascular function: a randomized, placebo-controlled study. J Clin Endocrinol Metab 2016;101:4205-4213

30. Manrique C, Habibi J, Aroor AR, et al. Dipeptidyl peptidase-4 inhibition with linagliptin prevents western diet-induced vascular abnormalities in female mice. Cardiovasc Diabetol 2016;15:94

31. Duvnjak L, Blaslov K. Dipeptidyl peptidase-4 inhibitors improve arterial stiffness, blood pressure, lipid profile and inflammation parameters in patients with type 2 diabetes mellitus. Diabetol Metab Syndr 2016;8:26

32. Koren S, Shemesh-Bar L, Tirosh A, et al. The effect of sitagliptin versus glibenclamide on arterial stiffness, blood pressure, lipids, and inflammation in type 2 diabetes mellitus patients. Diabetes Technol Ther 2012;14:561-567

33. Zografou I, Sampanis C, Gkaliagkousi E, et al. Effect of vildagliptin on hsCRP and arterial stiffness in patients with type 2 diabetes mellitus. Hormones 2015;14:118-125

34. Gordin D, Saraheimo M, Tuomikangas J, et al. Influence of posprandial hyperglycemic conditions on arterial stiffness in patients with type 2 diabetes. J Clin Endocrinol Metab 2016;101(3):1134-1143

35. Jackson EK, Mi Z, Tofovic SP, Gilleapie DG. Effect of dipeptidil peptidase 4 inhibition on arterial blood pressure is context dependent. Hypertension 2015;65:238-249

36. Barbieri M, Rizzo MR, Marfella R**,** et al. Decreased carotid atherosclerotic process by control of daily acute glucose fluctuations in diabetic patients treated by DPP-IV inhibitors. Atherosclerosis 2013;1-6

37. Ishikawa S, Shimano M, Watarai M, et al. Impact of sitagliptin on carotid intima-mdia thickness in patients with coronary artery disease and impaired glucose tolerance or mild diabetes mellitus. Am J Cardiol 2014;114:384-388

38. Mita T, Katakami N, Yoshii H, et al. Alogliptin, a dipeptidyl peptidase 4 inhibitor, prevents the progression of carotid atherosclerosis in patients with type 2 diabetes: the study of preventive effects of alogliptin on diabetic atherosclerosis (SPEAD-A). Diabetes Care. 2016;39:139-148

39. Mita T, Katakami N, Shiraiwa T, et al. Sitagliptin attenuates the progression of carotid intima-media thickening in insulin treated patients with type 2 diabetes: the sitagliptin preventive study of intima-media thickness evaluation (SPIKE). A randomized controlled trial. Diabetes Care 2016;39:455-464

40. Alam Md A, Chowdhury MRH, Jain P, et al. DPP-4 inhibitor sitagliptin prevents inflammation and oxidative stress of heart and kidney in two kidney and one clip (2K1C) rats. Diabetol Metab Syndr 2015;7:107

Table 1 – Clinical and Biochemical Parameters of the subjects

	Vildagliptin group (n=25)		Glibenclamide group (n=25)	
	Baseline	12 weeks	Baseline	12 weeks
Female, n (%)	17 (68)		15 (60)	
Age, years	60.5 ± 7.0		59.4 ± 8.2	
Duration diabetes, month	82.5 ± 67.7		71 ± 47.6	
Duration HT, month	114.2 ± 55		91.5 ± 52.2	
Body mass index, Kg/m^2	31.5 ± 3.3	31.4 ± 3.5	30 ± 3.5	29.8 ± 3.4
Waist circumference, cm	104.4 ± 6.5		101.1 ± 8.5	
Systolic BP, mmHg	137.5 ± 18.9	134 ± 20.7	133.2 ± 18.4	134 ± 21
Diastolic BP, mmHg	79.9 ± 8.9	79 ± 12.5	77.9 ± 9.2	78.7 ± 12.3
Fasting glucose, mg/dL	166 ± 38.7	147.5 ± 42.6*	164 ± 43.5	139 ± 54*
Fasting glucose, mmol/L	9.21 ± 2.15	8.19 ± 2.36*	9.10 ± 2.14	7.71 ± 2.99*
HbA1c (%)	8.3 ± 1.0	8.0 ± 1.34	7.9 ± 0.9	7.5 ± 1.4
HbA1c, mmol/L	67 ± 10.9	64 ± 14.6	63 ± 9.8	58 ± 15.3
Total cholesterol, mmol/L	4.81 ± 0.93	4.09 ± 0.77*	4.75 ± 0.87	4.47 ± 0.99
LDL cholesterol, mmol/L	2.74 ± 0.77	2.05 ± 0.65*	2.40 ± 0.76	2.38 ± 0.79
HDL cholesterol, mmol/L	1.13 ± 0.31	1.15 ± 0.31	1.12 ± 0.32	1.16 ± 0.31
Triglycerides, mmol/L	1.96 ± 1.03	1.77 ±0.99*	2.50 ± 1.26	2.22 ± 1.25
eGFR, $ml/min/1.73\ m^2$	86.2 ± 16	85.3 ± 14.4	91.2 ± 17.5	88.6 ± 14.7
ALT (U/L)	21.8 ± 9	20 ± 6.2	24.8 ± 10.7	24.3 ± 12
Sulphonylurea, n (%)	11 (44)		17 (68)	
Metformin, n (%)	25 (100)	24	25 (100)	24
Diuretic, n (%)	16 (64)	17 (70.8)	9 (36)	13 (54.2)
ACE inhibitor, n (%)	5 (20)	4 (16.6)	9 (36)	7 (29.2)
ARB, n (%)	19 (76)	19 (79.2)	15 (60)	17 (70.8)
CCB, n (%)	6 (24)	5 (20.8)	3 (12)	2 (8.3)
Statin, n(%)	13 (52)	16 (66.7)	15 (60)	17 (70.8)
Fibrate, n (%)	4 (16)	5 (20.8)	2 (8)	2 (8.3)
Aspirin, n (%)	3 (12)	3 (12.5)	10 (40)	10 (41.6)**

HT: hypertension, BP: blood pressure, HbA1c: glycated haemoglobin, LDL: low-density lipoprotein, HDL: high-density lipoprotein, eGFR: estimated glomerular filtration rate, ALT: alanine aminotransferase, ACE: angiotensin converting enzyme, CCB: calcium channel blocker, ARB: angiotensin receptor blocker

*P <0.05 within group

** P <0.05 between groups

Table 2 – 24-h non-invasive ABPM by Mobil-O-Graph® 24-hour PWA monitor

	Vildagliptin group Baseline	12 weeks	Glibenclamide group Baseline	12 weeks
SBP 24-h	123.875± 12.5015	119.087±1.6771*	125.130±12.2002	117.273±8.0545*
DBP 24-h	73.917±9.4405	71.435±8.2947*	76.739 ± 7.6468	70.909 ± 7.0028*
PPP 24-h	50.000± 7.6272	47.652 ±8.1386*	48.696 ± 9.1673	46.364± 7.5941*
MBP 24-h	96.958 ± 10.3692	93.261 ± 9.0915*	98.773 ± 9.1492	92.136 ± 6.4829*
CPP 24-h	38.67 ± 6.631	37.52 ± 6.707	38.05 ± 8.437	36.95 ± 7.103
PWV m/s 24-h	8.583 ± 1.1776	8.461 ± 1.1804	8.459 ± 1.2949	8.076 ± 1.1781*
cSBP 24-h	114.292 ±11.0236	110.217± 10.7533	116.409 ±12.1916	109.015 ±7.4626*
AIx75 24-h	29.84± 6.147	29.67 ± 6.339	30.08 ±6.866	30.03± 7.047

SBP: systolic blood pressure, DBP: diastolic blood pressure, PPP: periferic pressure pulse, MBP: Mean blood pressure, CPP: central pressure pulse, PWV: pulse wave velocity, cSBP: central systolic blood pressure, AIx75: heart rate corrected augmentation index, by 24-h blood pressure record (mobil).

*P <0.05

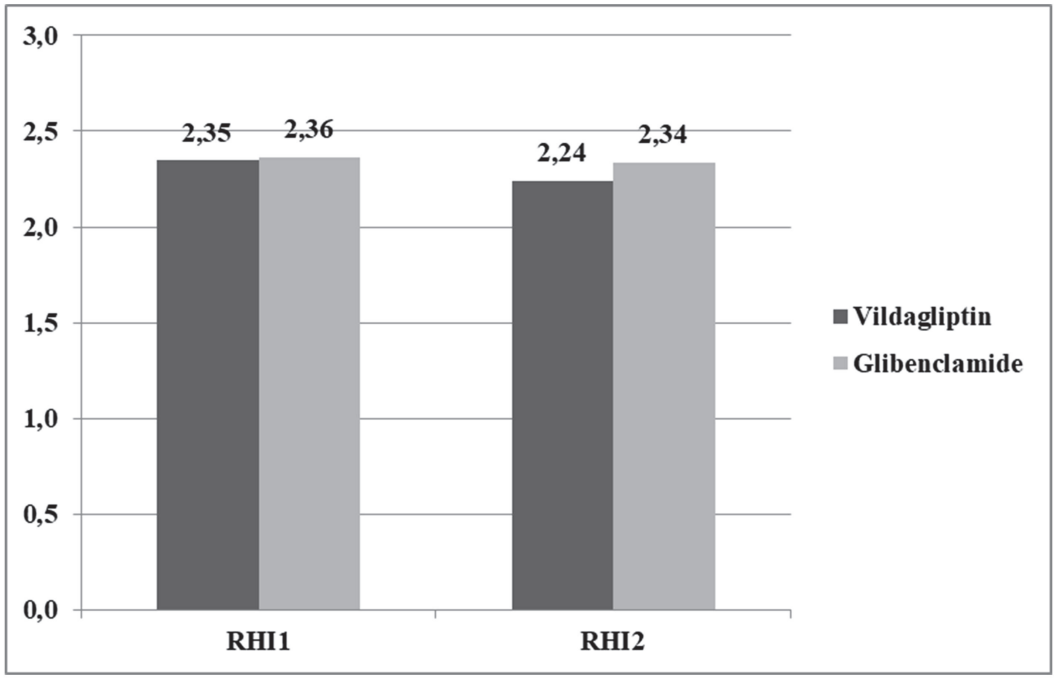

Figure 1 – Values of RHI 1 (baseline) and 2 (12 weeks after treatment) in the vildagliptin and glibenclamide groups. P > 0.05 within and between groups.

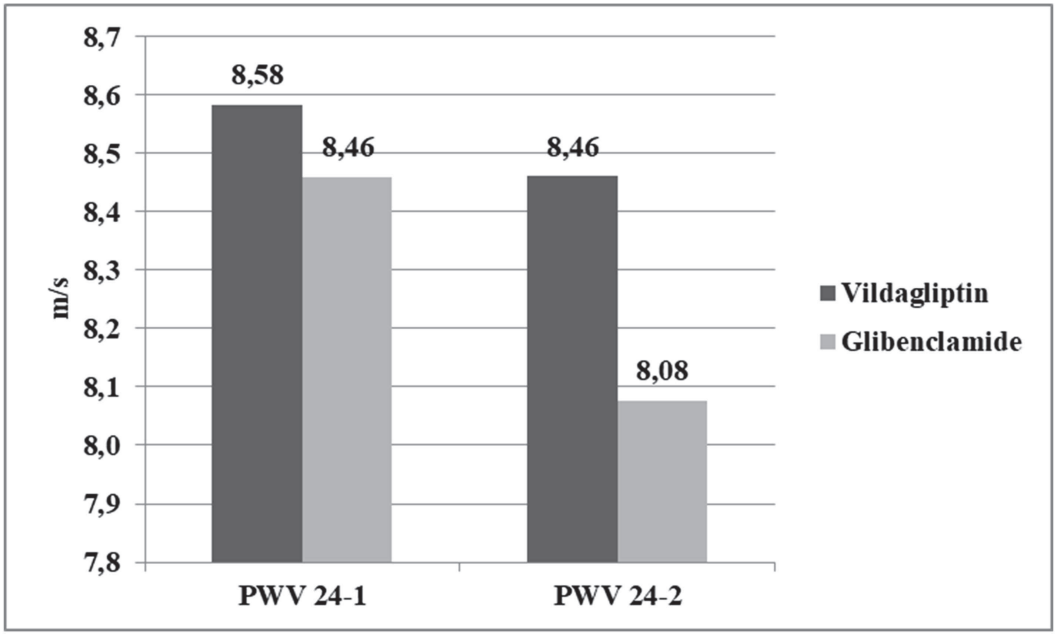

Figure 2 – Values of PWV 1 (baseline) and 2 (12 weeks after treatment). Although there was change in the glibenclamide group (P < 0.05), there was not difference comparing with vildagliptin group (P > 0.05).

3. CONCLUSÃO

3. CONCLUSÕES

Os resultados do presente estudo sugerem as seguintes conclusões:

- A função endotelial, avaliada pelo índice de hiperemia reativa (IHR), não foi diferente no estado basal entre os dois grupos e não melhorou em nenhum grupo, após 12 semanas de tratamento.
- A rigidez arterial, avaliada pela tonometria arterial, não sofreu influência da vildagliptina.
- A melhora do controle pressórico foi observada nos dois grupos, sendo considerada como mérito da terapia anti-hipertensiva.
- A vildagliptina mostrou neutralidade em relação aos efeitos vasculares, fato que pode ser indicativo de sua segurança em pacientes com DCV.
- Embora os métodos utilizados nesta pesquisa para avaliar a função endotelial e a rigidez arterial foram padronizados e amplamente estudados, é possível que diferentes métodos promovam diferentes resultados.
- Novos estudos são necessários para confirmar a ação favorável da vildagliptina e de outros fármacos do grupo de inibidores de DPP-4 e agonistas/análogos do GLP-1 na função endotelial.

4. REFERÊNCIAS BIBLIOGRÁFICAS

4. REFERÊNCIAS BIBLIOGRÁFICAS

1. Ministry of Health. DATASUS. Information about health: cardiovascular morbidity and mortality. http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sih/cnv/niuf.def Accessed 02 Dec 2014.
2. Heart Disease and Stroke Statistics - 2014 Update: A Report From the American Heart Association. Circulation. 2013;127:e6-e245.
3. Cipullo JP, Martin JF, Ciorlia LA, Godoy MR, Cação JC, Loureiro AA, et al. Hypertension prevalence and risk factors in a Brazilian urban population Arq Bras Cardiol 2010;94:519-26.
4. Bosi PL, Carvalho AM, Contrera D, Casale G, Pereira MA, Gronner MF, et al. Prevalence of diabetes and impaired glucose tolerance in the urban population of 30 to 79 years of the city of São Carlos, São Paulo. Arq Bras Endocrinol Metab. 2009;53:726-32.
5. Moraes SA, Freitas IC, Gimeno SG, Mondini L. Diabetes mellitus prevalence and associated factors in adults in Ribeirão Preto, São Paulo, Brazil, 2006: OBEDIARP Project. Cad Saúde Pública. 2010;26:929-41.
6. Ogurtsova K, Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017;128:40-50.
7. Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies.

Lancet 2010;375:2215-22.

8. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287:1420-26.

9. Penny WF, Ben-Yehuda O, Kuroe K, Long J, Bond A, Bhargava V, et al. Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol. 2001;37:766-74.

10. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, David H, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101:948-54.

11. Perrone-Filardi P, Cuocolo A, Brevetti G, Silvestro A, Storto G, Dellegrottaglie S, et al. Relation of brachial artery flow-mediated vasodilatation to significant coronary arterial disease in patients with peripheral arterial disease. Am J Cardiol. 2005;96:1337-41.

12. Mäkimattila S, Virkamäki A, Groop PH, Cockcroft J, Utriainen T, Fagerudd J, et al. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. Circulation. 1996;94:1276-82.

13. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368:1696-1705.

14. Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, Roman RJ. Antihypertensive effect of glucagons-like peptide 1 in Dahl salt-sensitive rats. J Hypertens. 2003;21:1125-35.

15. Nyström T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahrén B, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary arterial disease. Am J Physiol Endocrinol Metab. 2004;287:E1209-15.

16. Ceriello A, Esposito K, Testa R, Bonfigli AR, Marra M, Giugliano D. The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an "endothelial resistance" to the glucagon-like peptide 1 in diabetes. Diabetes Care. 2011;34:697-702.

17. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Druker DJ, Husain M. Cardioprotective and vasodilatadory actions of glucagons-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and – independent pathways. Circulation. 2008;117:2340-50.

18. Gurkan E, Tarkun I, Sahin T, Cetinarslan B, Canturk Z. Evaluation of exenatide versus insulin glargine for the impact on the endothelial functions and cardiovascular risk markers. Diabetes Res and Clin Pract. 2014;106:567-75.

19. Forst T, Michelson G, Ratter F, Weber MM, Anders S, Mitry M, et al. Addition of liraglutide in patients with Type 2 diabetes well controlled on metformin monotherapy improves several markers of vascular function. Diabet Med. 2012;29:1115-8.

20. Gejl M, Sondergaard HM, Stecher C, Bibby BM, Moller N, Botker HE, et al. Exenatide alters myocardial glucose transport uptake and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. J Clin Endocrinol Metab. 2012;97:E1165-69.

21. Subaran SC, Sauder MA, Chai W, Jahn LA, Fowler DE, Aylor KW, et al. GLP-1

at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. Clin Sci (Lond). 2014;127:163-70.

22. Faber R, Zander M, Pena A, Michelsen MM, Mygind ND, Prescott E. Effect of glucagon-like peptide-1 analogue liraglutide on coronary microvascular function in patients with type 2 diabetes – a randomized, single-blinded, cross-over pilot study. Cardiovascular Diabetol. 2015;14:41.

23. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal derived factor-1 alpha. Diabetes Care. 2010;33:1607-9.

24. Van Poppel PC, Netea MG, Smits P, Tack CJ. Vildagliptin improves endotheliumdependent vasodilatation in type 2 diabetes. Diabetes Care. 2011;34:2072-7.

25. Maruhashi T, Higashi Y, Kihara Y, Yamada H, Sata M, Ueda S, et al. Long-term effect of sitagliptin on endothelial function in type 2 diabetes: a sub-analysis of the PROLOGUE study. Cardiovasc Diabetol. 2016;15:134.

26. Ayaori M, Iwakami N, Uto-Kondo H, Sato H, Sasaki M, Komatsu T, et al. Dipeptidyl peptidase-4 inhibitors attenuate endothelial function as evaluated by flow-mediated vasodilatation in type 2 diabetic patients. J Am Heart Assoc. 2013; 2:e003277.

27. Scirica BM, Bhatt DL, Braunwald E, Steg G, Davidson J, Hirshberg B, et al, for the SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. N Engl J Med. 2013;369:1317-26.

28. White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, et al, for the EXAMINE Investigators. Alogliptin after Acute Coronary Syndrome in Patients with Type 2 Diabetes. N Engl J Med. 2013;369:1327-35.

29. Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, et al. TECOS Study Group. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2015;373:232-42.

30. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J. 2003;146:168-74.

31. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, et al. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. J Am Coll Cardiol. 2003;41:1761-68.

32. Bonetti PO, Pumper GM, Higano ST, Holmes DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol. 2004;44:2137-41.

33. Kuvin JT, Mammen A, Mooney P, Alsheikh-Ali AA, Karas Rh. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vasc Med . 2007;12:13-6.

34. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. J Appl Physiol. 2006;101:545-8

35. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. J Am Heart Assoc.

2015;4:e002270

36. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation. 2004;109:184-9.

37. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension. 2007;49:1202-6.

38. Tedesco MA, Natale F, Di Salvo G, Caputo S, Capasso M, Calabró R. Effects of coexisting hypertension and type II diabetes mellitus on arterial stiffness. J Hum Hypertens. 2004;18:469-73.

39. Gedikli O, Kiris A, Yilmaz H, Ozturk S, Baykan M, Durmus I, et al. The relationship between endothelial damage and aortic augmentation index. Clin Exp Hypertens. 2010;32:29-34.

40. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113:657-63.

41. Mulè G, Cottone S, Cusimano P, Riccobene R, Palermo A, Geraci C, et al. The association of microalbuminuria with aortic stiffness is independent of c-reactive protein in essential hypertension. Am J Hypertens. 2009;22:1041-7.

42. Hermans MM, Henry R, Dekker JM, Kooman JP, Kostense PJ, Nijpels G, et al. Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness: The Hoorn study. J Am Soc Nephrol. 2007;18:1942-52.

43. Takenaka T, Mimura T, Kanno Y, Suzuki H. Qualification of arterial stiffness as a risk factor to the progression of chronic kidney disease. Am J Nephrol.

2005;25:417-24.

44. Nürnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schäfers RF. Augmentation index is associated with cardiovascular risk. J Hypertens. 2002;20:2407-14.

45. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation. 2006;113:664-70.

46. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55:1318-27.

47. DeLoach SS, Townsend RR. Vascular stiffness: its measurements and significance for epidemiologic and outcome studies. Clin J Am Soc Nephrol. 2008;3:184-92.

48. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation. 2006;113:1213-25.

49. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. Hypertension. 2007;50:197-203.

50. Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guvonvarc'h PM, et al. Central pulse pressure and mortality in end-stage renal disease. Hypertension. 2002; 39:735-38.

51. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in human. J Physiol. 2000;525:263-70.

52. Balduino Mendes AB, Giollo-Junior LT, de Andrade DO, Gregorio ML, Yugar Toledo JC, Vilela-Martin JF. How to investigate the vascular changes in resistant hypertension. Curr Hypertens Rev. 2016;12:139-47.

53. Barbaro NR, Fontana V, Moelolo R, de Faria AP, Sabbatini AR, Fonseca FH, et al. Increased arterial stiffness in resistant hypertension is associated with inflammatory biomarkers. Blood Press. 2015;24:7-13.

54. Wascher TC, Boes U. Forearm vascular reactivity is differentially influenced by gliclazide and glibenclamide in chronically treated type 2 diabetic patients. Clin Physiol Funct Imaging. 2005;25:40-6.