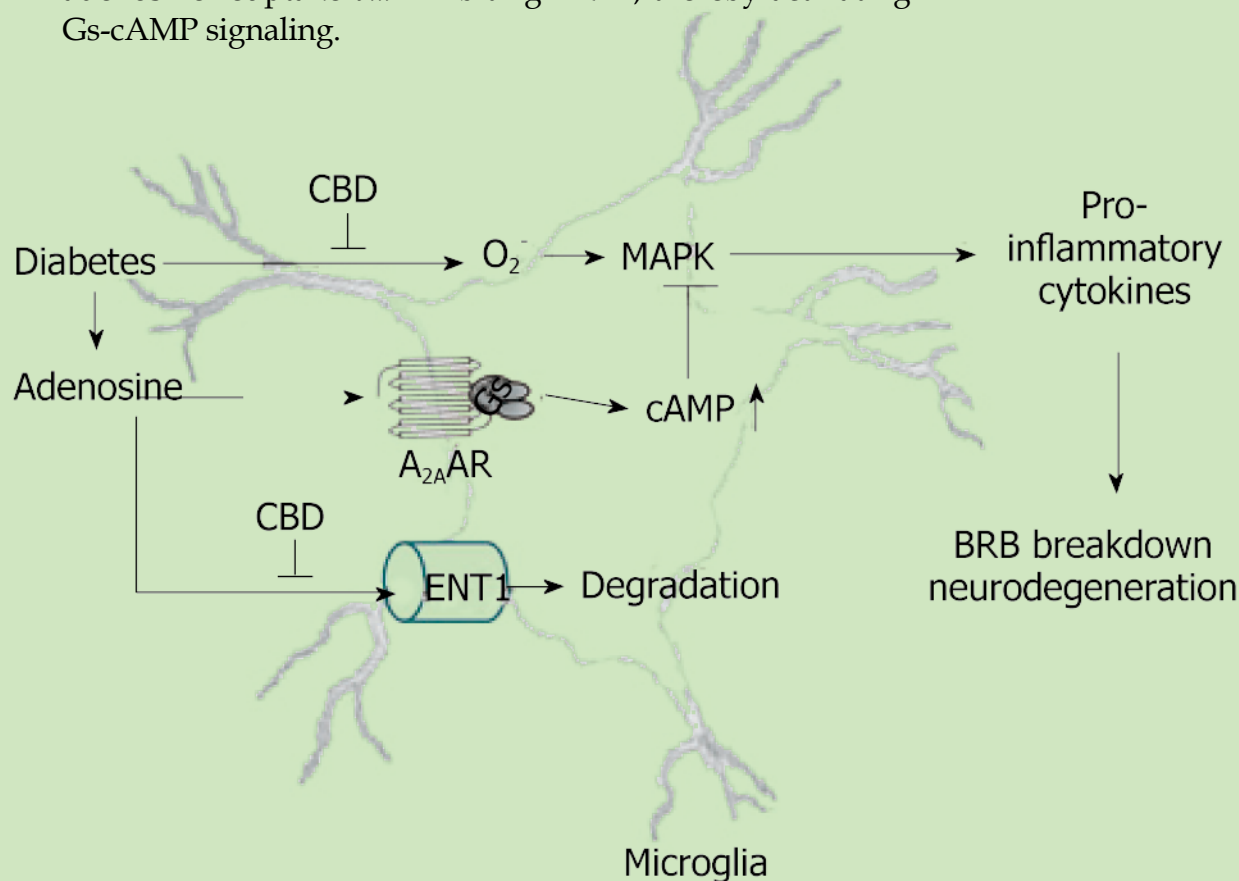


The hypothetical mechanism of anti-inflammation effect CBD in diabetic retinopathy. Diabetes causes release of adenosine and pro-inflammatory cytokines *via* superoxide formation and MAPK activation, leading to DR. Adenosine-initiated anti-inflammation *via* A_{2A}AR-Gs-cAMP signaling is terminated rapidly due to adenosine reuptake by equilibrative nucleoside transporter (ENT) and subsequent metabolism. CBD blocks superoxide formation and inhibits adenosine reuptake *via* inhibiting ENT1, thereby activating A_{2A}AR-Gs-cAMP signaling.



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Contents

Bimonthly Volume 1 Number 1 March 15, 2010

EDITORIAL

- 1 What is the purpose of launching *World Journal of Diabetes*?
Ma LS
- 3 Systemic and metabolic effects of PDE5-inhibitor drugs
Aversa A

GUIDELINES FOR CLINICAL PRACTICE

- 8 Combination drug treatment in obese diabetic patients
Filippatos TD, Elisaf MS

REVIEW

- 12 Diabetic retinopathy: Role of inflammation and potential therapies for anti-inflammation
Liou GI

BRIEF ARTICLES

- 19 Effect of vildagliptin as add-on therapy to a low-dose metformin
Filozof C, Schwartz S, Foley JE

Contents

World Journal of Diabetes
Volume 1 Number 1 March 15, 2010

ACKNOWLEDGMENTS I Acknowledgments to reviewers of *World Journal of Diabetes*

APPENDIX I Meetings
I-V Instructions to authors

ABOUT COVER Liou GI. Diabetic retinopathy: Role of inflammation and potential therapies for anti-inflammation.
World J Diabetes 2010; 1(1): 12-18
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The major task of *WJD* is to report rapidly the most recent results in basic and clinical research on diabetes including: metabolic syndrome, functions of α , β , δ and PP cells of the pancreatic islets, effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. This covers epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics. Reports on new techniques for treating diabetes are also welcome.

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Room 903, Building D, Ocean International Center,
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Telephone: 0086-10-8538-1892
Fax: 0086-10-8538-1893
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<http://www.wjnet.com>

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Fax: 0086-10-8538-1893
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Room 903, Building D, Ocean International Center,
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What is the purpose of launching *World Journal of Diabetes*?

Lian-Sheng Ma

Lian-Sheng Ma, Beijing Baishideng BioMed Scientific Co., Ltd., Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China

Author contributions: Ma LS solely contributed to this paper.
Correspondence to: Lian-Sheng Ma, Professor, President and Editor-in-Chief, Beijing Baishideng BioMed Scientific Co., Ltd., Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China. l.s.ma@wjgnet.com

Telephone: +86-10-59080036 Fax: +86-10-85381893

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Abstract

The first issue of *World Journal of Diabetes (WJD)*, whose preparatory work was initiated on September 23, 2008, is published on March 15, 2010. The *WJD* Editorial Board has now been established and consists of 323 distinguished experts from 38 countries. Our purpose of launching *WJD* is to publish peer-reviewed, high-quality articles via an open-access online publishing model, thereby acting as a platform for communication between peers and the wider public, and maximizing the benefits to editorial board members, authors and readers.

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Key words: Maximization of personal benefits; Editorial board members; Authors; Readers; Employees; *World Journal of Diabetes*

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INTRODUCTION

I am very pleased to announce that the first issue of

World Journal of Diabetes (World J Diabetes, WJD), online ISSN 1948-9358, DOI: 10.4239 is published on March 15, 2010. Originally, the journal was titled *Diabetes Review Letters* when preparatory work was initiated on September 23, 2008. The *WJD* Editorial Board has now been established and consists of 323 distinguished experts from 38 countries.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. To realize these desired attributes of a journal and create a well-recognized journal, the following four types of personal benefits should be maximized.

MAXIMIZATION OF PERSONAL BENEFITS

The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others.

Maximization of the benefits of editorial board members

The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution.

Maximization of the benefits of authors

Since *WJD* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJD* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading.

Maximization of the benefits of readers

Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion^[1].

Maximization of the benefits of employees

It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal^[2,3]. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJD* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

SCOPE

The major task of *WJD* is to rapidly report the most recent results in basic and clinical research on diabetes research fields, specifically including metabolic syndrome, including functions of α , β , δ and PP cells of the pan-

creatic islet, the effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. The specialties cover epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics.

COLUMNS

The columns in *WJD* will include: (1) Editorial: to introduce and comment on major advances in rapidly developing areas and their importance; (2) Frontier: to review recent developments, comment on current research status in important fields, and propose directions for future research; (3) Topic Highlight: this column consists of three formats, including: (a) 10 invited review articles on a hot topic; (b) a commentary on common issues associated with this hot topic; and (c) a commentary on the 10 individual articles; (4) Observation: to update the development of old and new questions, highlight unsolved problems, and provide strategies for their resolution; (5) Guidelines for Basic Research: to provide Guidelines for basic research; (6) Guidelines for Clinical Practice: to provide guidelines for clinical diagnosis and treatment; (7) Review: to review systemically the most representative progress and unsolved problems, comment on current research status, and make suggestions for future work; (8) Original Article: to report original and innovative findings; (9) Brief Articles: to report briefly on novel and innovative findings; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: to discuss and reply to contributions published in *WJD*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: to introduce and comment on quality monographs; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities on basic research and clinical practice.

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Systemic and metabolic effects of PDE5-inhibitor drugs

Antonio Aversa

Antonio Aversa, Department of Medical Pathophysiology, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy

Author contributions: Aversa A solely contributed to this paper. Correspondence to: Antonio Aversa, MD, PhD, Professor of Sexual Medicine, Department of Medical Pathophysiology, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy. antonio.aversa@uniroma1.it

Telephone: +39-06-49970721 Fax: +39-06-4461450

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Abstract

Phosphodiesterase type-5 inhibitor (PDE5-i) drugs were first marketed in 1998 (sildenafil) for 'on-demand' treatment of male erectile dysfunction (ED) of any origin. They selectively inhibit intrapenile PDE5 isoenzyme which in turn increases intracellular cyclic guanosine monophosphate levels, thus resulting in prolonged relaxation of cavernosum smooth muscle cells and facilitating the erectile process. Since 2003, two new molecules (tadalafil and vardenafil) have been introduced, resulting in greater interest in these compounds and leading patients to ask for more prescriptions from their doctors. The vast use of PDE5-i in diabetic and cardiovascular ED patients led researchers to investigate their possible extra sexual effects. Several studies investigating their effects on endothelium, coronary and pulmonary circulation, inferior oesophageal sphincter and kidney functions have appeared and, finally, sildenafil was approved for the treatment of pulmonary arterial hypertension. Recent animal studies highlighted a possible interaction between chronic PDE5 inhibition and glucose homeostasis which occurs through a marked improvement of high fat diet induced insulin resistance. If this data is extended to humans, a new scenario will be opened for the chronic use of PDE5-i for sexual rehabilitation along with cardiovascular and metabolic benefits.

INTRODUCTION

Erectile dysfunction (ED) is the persistent inability to achieve and maintain an erection adequate for satisfactory sexual performance^[1]. Its prevalence is underestimated because the patients treated (less than 20% out of total) are considered as only the 'tip of the iceberg'^[2]. The probability of ED increases with ageing and the presence of diabetes mellitus, hypertension, hypercholesterolemia, ischemic cardiac disease, depression and obesity. Although cigarette smoking is not a direct causative factor, it may increase the risk of presenting with peripheral vascular disease and hypertension. Drugs and alcohol abuse may also increase the risk of ED. Based on our data, as more than 70% of the male population affected by moderate to severe ED complain of concomitant diseases, we conclude that it is a symptom and not a disease itself^[3]. The probability of manifested severe ED increases two-fold in the presence of the above mentioned diseases, thus suggesting that modification of associated risk factors may contribute to improve ED in internal medicine patients.

Phosphodiesterase type-5 inhibitors (PDE5-i) are currently used in the treatment of male ED. Sildenafil, vardenafil and tadalafil all inhibit PDE5 at the level of the corpus cavernosum^[4] with different onset of action, bioavailability and pharmacokinetic profiles (Table 1)^[5]. However, tadalafil is innovative due to its longer half-life

(17.5 and proven efficacy rates after 36 h) and highest selectivity (Table 2). These molecules are contraindicated in the presence of class New York Heart Association III /IV cardiac disease and in all men consuming nitric oxide (NO)-derivatives in any pharmaceutical form. In the remaining cases, they can be used safely with an overall response rate of approximately 70%-80%; adverse events are dose-related, mild-moderate in intensity (mainly headache 15%-20%, gastric discomfort 10% and rhinitis 5%-10%) and tend to disappear over the time^[6]. Since tadalafil has a longer half-life but similar onset of action when compared to sildenafil and vardenafil, we can assume that it can be considered the ideal drug for men who do not wish to be tied to pill consumption for sexual activity, thus contributing to the elimination of the state of performance-induced anxiety frequently encountered in almost all sexual dysfunctions.

SYSTEMIC EFFECTS OF PDE5 INHIBITORS

Since PDE5-i is highly efficacious and widely used in treating male ED, it is a timely hypothesis that it may exert important systemic effects at the endothelial level^[7]. To further investigate this, several studies have investigated the effects of sildenafil on blood pressure variations mainly due to its vasodilatory effects and possible interactions with anti-hypertensive drugs^[8]. Sildenafil did not decrease systemic arterial pressure because of its effect on renal PDE5 inactivation which elicits renin production, thus counterbalancing hypotensive effects^[9]. Also, subsequent confirmatory studies carried out in men with liver cirrhosis and ascites suggested the activation of the renin-angiotensin-aldosterone pathway as a possible regulatory mechanism^[10]. Further studies in an animal cirrhosis model clearly concluded that both acute and chronic sildenafil administration has a natriuretic effect, helping to prevent water retention associated with liver cirrhosis^[11]. The recent approval of sildenafil for the treatment of pulmonary arterial hypertension (PAH) opens new applications for this class of drug (Figure 1), considering its efficacy in such a difficult-to-treat population usually refractory to conventional therapies^[12]. Recently, new acquisition data revealed that tadalafil^[13] and vardenafil^[14] are equally safe and effective for the treatment of PAH. In a rat model of chronic NO deprivation where hypertension and aggravation of post-ischemic ventricular dysfunction are associated with loss of vascular endothelium-relaxant function, sildenafil provided significant cardiovascular protection, primarily by maintaining tissue cyclic guanosine monophosphate (cGMP) levels^[15,16]. It is important to remember that sildenafil efficacy has been already demonstrated in idiopathic refractory achalasia^[17] and Raynaud's phenomenon^[18]. Tadalafil has also been demonstrated to be effective in improving Raynaud's attacks in men with systemic sclerosis^[19]. However, because NO promotes upper airway congestion, muscle relaxation and pulmonary

Table 1 Pharmacological profile of oral PDE5-i at therapeutic dosage for ED

Parameter	Sildenafil 100 mg	Tadalafil 20 mg	Vardenafil 20 mg
T _{max} (h)	1.16 ± 0.99	2	0.66 (0.250-3.0)
T _{1/2} (h)	3.82 ± 0.84	17.5	3.94 ± 1.31
C (max ng/m)	327 ± 236	378	20.9 ± 1.83
AUC (ng × h/m)	1963 ± 859	8066	74.5 ± 1.82

PDE5-i: Phosphodiesterase type-5 inhibitor; ED: Erectile dysfunction.

Table 2 *In vitro* enzymatic inhibition activity of the marketed PDE5-i (IC₅₀ nmol/L)

PDE isoform	Sildenafil	Tadalafil	Vardenafil
PDE-1	60	> 10 000	257
PDE-2	> 10 000	> 10 000	> 10 000
PDE-3	2 600	> 10 000	3 600
PDE-4	1 800	> 10 000	5 700
PDE-5	3.8	1	0.7
PDE-6	7.4	780	15.7

Table 3 Causes and main interventions in endothelial dysfunction

Factors associated with endothelial dysfunction	Interventions to correct endothelial dysfunction
Aging	L-arginine
Male sex	Estrogens
Cigarette smoking	Smoking cessation
History of CHD	Antioxidants
Low HDL- and high LDL-COL	Statins
Hypertension	ACE-i
Hyperhomocysteinemia	Homocysteine lowering (folates)
Diabetes/obesity	Exercise
Erectile dysfunction	PDE5-i

vasodilatation, it is remarkable that in patients with severe obstructive sleep apnoea, a single 50-mg dose of sildenafil at bedtime worsens respiratory and desaturation events^[20].

Since endothelial dysfunction plays a key role in the pathogenesis of the atherosclerotic process, growing interest in the effects of PDE5-i in preventing atherosclerosis and the vascular endothelium damage has developed^[21]. The event that triggers endothelial dysfunction is represented by the reduction of overall antioxidant pool with the consequent reduced response to oxidative stress and the activation of several pro-atherogenic processes^[22] (Table 3): (1) reduction of NO bioavailability; (2) increased levels of circulating free fatty acids with subsequent sub-endothelial storage of lipid depots; and (3) increased smooth muscle cell proliferation of the media layer of the vascular wall^[23]. Pro-inflammatory and infective processes may in turn contribute to activate and amplify the acute endothelial injury, thus perpetuating a vicious circle. In early atherogenic lesions, endothelial dysfunction causes adhesion and migration of monocytes and T-lymphocytes in the vascular inner layer in response to increased end-

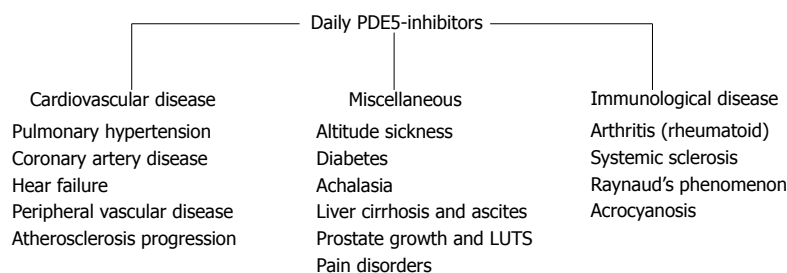


Figure 1 Potential applications of PDE5 inhibitors in internal medicine.

othelial production of intercellular molecules, i.e. selectin, Vascular Cellular Adhesion Molecule-1 and Inter-Cellular Adhesion Molecule-1 (ICAM-1)^[24]. Although data regarding the effects of PDE5-i on endothelial function are scarce, pilot studies using chronic administration of sildenafil have shown improvements both in endothelial^[25] and cardiac functions^[26]. Subsequent studies with an alternative dosing regimen of tadalafil (every other day) confirmed patients' preference in the treatment of ED^[27]. By using a once-a-day dosing in initially non-responders to on-demand treatment, tadalafil was able to restore potency in more than 30%^[28]. In another study by Porst *et al*^[29], daily tadalafil at low dosages (5 and 10 mg) was safe and effective with almost 50% of men and produced normalization of erectile function. These benefits were confirmed by our group in a recent study in men with ED of broad etiology^[30]. In that study, it was demonstrated that the rehabilitative effects of chronic tadalafil on vascular function occurs *via* improvement of surrogate markers of endothelial function, i.e. C-reactive protein, endothelin-1 and ICAM-1. This led to marked improvement of flow-mediated-vasodilatation (FMD) at the level of the cavernous arteries as well as better inflow to the penis, as shown by ultrasound technique, when compared with on-demand regimes. Moreover, this study was the first one to report consistent improvements in morning erections as a possible mechanism for rehabilitative effects which persisted even after 15 d withdrawal. The same group confirmed those results in men without ED and increased cardiovascular risk^[31]. Another possible explanation of increased endothelial function comes from pioneer studies suggesting a direct effect of PDE5-i on modulating the number of circulating endothelial progenitor cells (EPCs). In men with ED, this number is reduced compared with controls and studies have demonstrated that chronic tadalafil significantly increases EPCs^[32], thus leading to improvements in brachial FMD. Overall, these data give the rationale for the chronic use of PDE5-i in men with ED and cardiovascular risk factors^[33]. Attention has been given to the endocrine effects of PDE5-i administration. In a recent study^[34], the effects of the administration of sildenafil and tadalafil on steroid hormones were assessed for three months in 80 patients with ED. Total and free testosterone rose significantly in subjects using tadalafil, probably due to a higher number of sexual intercourses per month allowed by the long-acting drug, speculated to cause an indirect and sustained activation of the hypothalamic-pituitary-testicular axis. However, our studies demonstrated that this rise is lost after 12 mo

of continuous tadalafil consumption (3 pills per wk)^[35]. Subsequent studies carried out in human adipocytes *ex vivo* suggest that, during acute exposure to tadalafil, an aromatase activation occurs that causes an increase in estradiol concentrations (data not published) that may be responsible for vascular beneficial effects of the drug. Thus, the hypothesis that tadalafil may modulate aromatase activity in humans during acute and chronic administration highlights potential implications for the treatment of prostate and breast cancer. Evidence-based medicine with off-label use of PDE5 inhibitors is scarce but pilot studies suggest that chronic PDE5-i use determines better endothelial function and may slow down progression of atherosclerosis, especially in men with organic ED and endothelial dysfunction at baseline. This latter effect may be a consequence of improvements in FMD (brachial and cavernous arteries), in biomarkers of endothelial function, in the number of EPCs and finally, in testosterone/estradiol ratio^[36].

METABOLIC EFFECTS OF PDE5 INHIBITORS

Besides the above mentioned vascular systemic effects, emerging data suggest that PDE5-i may also impact on metabolic parameters. It is known that NO plays an important role in mediating insulin-induced effects, especially on glucose uptake at the muscular level^[37]. Knock-out mice models for endothelial NO-synthase (eNOS) are insulin-resistant^[38,39] and their treatment with drugs that inhibit NO formation produces inhibitory effects on insulin-mediated glucose uptake^[40]. Also, in a diabetic human model where eNOS synthesis is reduced, insulin-resistance frequently occurs^[41]. Given the important role of the NO-cGMP pathway in muscular metabolism, the effects of sildenafil on insulin function has been investigated in an animal model of insulin-resistance *in vivo*. Chronic sildenafil administration (12 wk) counteracted the detrimental effects of a high-fat diet on endothelial function and insulin resistance by improving energy balance and insulin function. This effect persisted even in the presence of NO donors, suggesting direct effects of sildenafil on metabolism other than eNOS activation^[42]. In this regard, a pilot study in humans has been carried out using tadalafil and it demonstrated that in ED men who are not insulin resistant, a 30% increase of basal insulin secretion occurs^[28]. However, in that study, insulin sensitivity was not assessed. Overall, these

preliminary suggest a fascinating interplay between energy balance, insulin action and PDE5-i and may give new potential applications to this class of drugs (Figure 1).

CONCLUSION

11 years after the sildenafil launch, ED is still an under diagnosed and under treated condition in the internal medicine setting, probably as it is perceived by the patient as a stress or age related condition. ED alters self-esteem and quality of life for patients and their partners and should be considered a symptom of an underlying condition, which can often lead to a diagnosis of organic disease. Each physician should consider the close relationship between internal medicine diseases and the pathophysiology of ED and each intervention should be intended to modify lifestyle and concomitant drugs with anti-erectile properties. Taking preliminary data into account, it can be postulated that PDE5-i may exert systemic other than penile rehabilitative effects, as well as important effects on pulmonary, gastrointestinal and metabolic systems. However, extra-sexual applications are not evidence-based and cost-effective at the moment. In the near future, the approval of new PDE5-i molecules will allow reduced costs related to investigational protocols targeted for a larger use of these drugs.

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Combination drug treatment in obese diabetic patients

Theodosios D Filippatos, Moses S Elisaf

Theodosios D Filippatos, Moses S Elisaf, Department of Internal Medicine, University of Ioannina, Ioannina 45110, Greece

Author contributions: Filippatos TD prepared and wrote the editorial; Elisaf MS made corrections and did the final editing of the manuscript.

Correspondence to: Moses S Elisaf, MD, FRSH, FASA, FISA, Professor of Medicine, Department of Internal Medicine, School of Medicine, University of Ioannina, Ioannina 45110, Greece. egepi@cc.uoi.gr

Telephone: +30-2651-007509 Fax: +30-2651-007016

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Dr., 101 PSB, Auburn University, Auburn, AL 36849, United States

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Abstract

Drug combinations that include antiobesity drugs (such as orlistat and sibutramine) and target cardiovascular disease (CVD) risk factors may be a good approach to patients with type 2 diabetes and/or metabolic syndrome (MetS). Our group has investigated the orlistat-fenofibrate combination treatment in obese patients with MetS and the orlistat-ezetimibe and the sibutramine-antihypertensive combination treatment in obese patients with hyperlipidaemia with promising results in CVD risk factor reduction. In these studies, the combination treatment significantly improved the lipid and lipoprotein profile, the carbohydrate metabolism parameters and many other variables playing a role in the atherosclerotic process. Small studies give promising results but double-blind, randomized trials examining the effects of such multifactorial treatment in hard CVD endpoints in diabetic or MetS patients are missing.

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Key words: Diabetes; Metabolic syndrome; Orlistat; Sibutramine; Fenofibrate; Ezetimibe; Weight loss

Peer reviewer: Suresh Mathews, PhD, Assistant Professor, Department of Nutrition and Food Sciences, 260 Lem Morrison

INTRODUCTION

The prevalence of metabolic syndrome (MetS) and type 2 diabetes (T2DM) is increasing and expected to rise in the next decade^[1-3]. Visceral obesity and insulin resistance (IR) play a central role in the pathogenesis of these conditions^[4]. IR results in hyperinsulinaemia and high levels of plasma free fatty acids which enter into the hepatocyte cytoplasm, resulting in the overproduction of very low density lipoprotein cholesterol (VLDL) particles by the liver^[5]. In patients with increased VLDL concentration (such as patients with MetS and T2DM), the cholesterol esters in low density lipoprotein (LDL) particles are exchanged for triglycerides (TGs) in VLDL by the cholesterol ester transfer protein. Then, triglycerides in LDL are hydrolyzed by hepatic lipase, producing small dense low density lipoprotein cholesterol (sdLDL) particles^[6]. Our group has shown that subjects with MetS exhibit significantly higher concentrations of the atherogenic sdLDL subfractions compared with non MetS individuals^[6]. Previous studies have reported a linear correlation between the concentration of sdLDL particles and the risk for the development of cardiovascular events^[7-9].

Diabetic patients have increased cardiovascular disease (CVD) morbidity and mortality which are in part associated with the high prevalence of visceral obesity, dyslipidemia and hypertension in this population^[10]. These patients may require a multifactorial approach targeting excess body weight and CVD risk factors to reduce CVD events. In this context, combinations of antiobesity drugs (such as orlistat and sibutramine)

and drugs that target CVD risk factors may offer an approach to lower cardiometabolic risk in such patients.

STUDIES INCLUDING ORLISTAT

Orlistat is an anti-obesity drug with a well documented efficacy in weight reduction and maintenance^[11-13]. The drug also has beneficial effects on metabolic indices, reducing the incidence of T2DM in patients with impaired glucose tolerance^[12-15]. It was also shown to decrease LDL-C levels to a greater degree than expected from weight loss alone^[12,13]. In obese patients with hypercholesterolaemia, orlistat - fluvastatin, orlistat - simvastatin and orlistat - cerivastatin combinations led to pronounced weight loss and a greater decrease in LDL-C concentration compared with statin monotherapy^[16-18].

Our group assessed in an open-label randomized study (the FenOrli study) the effect of orlistat and fenofibrate combination in overweight and obese patients ($n = 89$) with MetS [defined as having 3 of the following 5 criteria: waist circumference ≥ 102 cm in men or ≥ 94 cm in women, blood pressure $\geq 130/85$ mmHg (or antihypertensive treatment), HDL-C ≤ 40 mg/dL in men or ≤ 50 mg/dL in women, TG ≥ 150 mg/dL, glucose ≥ 126 mg/dL or antidiabetic treatment]^[19]. At the end of the 6-mo treatment period only 54% of patients in the orlistat group, 46% in the fenofibrate group and 29% in the combination group still met the MetS diagnostic criteria ($P < 0.01$ *vs* baseline in all treatment groups)^[20]. At 6 mo significantly greater reduction was observed in body weight, body mass index (BMI) and waist circumference in groups receiving orlistat^[20]. There were significantly greater reductions in plasma levels of total cholesterol (TC), LDL cholesterol (LDL-C) and TGs in the combination group compared with monotherapy. Glucose, insulin and homeostasis model assessment (HOMA) index levels were improved after the 6-mo treatment significantly more in groups receiving orlistat compared with fenofibrate monotherapy. We also observed significant reductions in blood pressure in all treatment groups^[20]. Furthermore, at 6 mo fenofibrate and combination treatment groups experienced a greater reduction in sdLDL-C levels (-63% and -77% respectively) along with a greater increase in LDL particle diameter compared with orlistat monotherapy (-35%, $P < 0.05$ for both)^[20], a result which may be clinically relevant since sdLDL particles are considered the most atherogenic^[9]. No significant alteration of small or large HDL-C plasma levels occurred with combined orlistat-fenofibrate treatment^[21].

Our group also investigated the effects of orlistat and ezetimibe combination in an open-label randomized trial in 86 overweight and obese patients with hypercholesterolemia^[22]. Significantly greater reductions were observed for BMI, waist circumference and body weight at 6 months in groups receiving orlistat compared with ezetimibe monotherapy. At the end of the 6-mo treatment period, significant reductions in LDL-C levels were

observed in all groups. The fall in LDL-C concentration was significantly greater in the combination group compared with either monotherapy^[22]. We also observed greater reductions in TC and TG concentration in the combination group compared with monotherapy. Glucose, insulin and HOMA index levels were improved after the 6-mo treatment significantly more in groups receiving orlistat. We also observed significant reductions in BP in groups receiving orlistat. The sdLDL-C concentration was reduced significantly more in the combination group compared with both monotherapies. In the orlistat-ezetimibe combination, HDL-2 subclass did not significantly change while the cholesterol concentration of HDL-3 subclass decreased significantly^[23].

STUDIES INCLUDING SIBUTRAMINE

Sibutramine is another antiobesity drug with a well established efficacy in weight reduction and maintenance of weight loss^[24]. Weight loss with sibutramine treatment has been associated with an improvement of insulin sensitivity and a favorable lipid profile^[25]. In a recent study we examined the effect of sibutramine together with verapamil slow release/trandolapril (VeTra) combination tablet *vs* VeTra alone in obese hypertensive patients^[26]. The combination treatment resulted in greater reductions of BP (significant only for diastolic BP) compared with the antihypertensive treatment alone at 6 mo with no significant change in heart rate in any group^[26]. Significant reductions in body weight, BMI and waist circumference were observed in both groups during the first 3 mo but only in the SiVeTra group at the end of the study. Significant reductions were noted in insulin levels and HOMA index in both groups but they were greater in the SiVeTra group compared with the VeTra group. We observed significant reductions in TC, TGs and LDL-C only in the SiVeTra group (all $P < 0.05$ *vs* VeTra group). Subfraction analysis of LDL and HDL particles was only performed in the SiVeTra group and showed a significant decrease in sdLDL-C concentration but no significant change in HDL particle distribution during treatment. Additionally, pre-beta1-HDL levels, a precursor of HDL particles, did not change significantly in the SiVeTra group. We observed significant reductions in visfatin (an adipokine related with atherosclerotic diseases^[27]) and high sensitivity C-reactive protein plasma levels at the end of the 6-month treatment in the SiVeTra group.

STUDIES INCLUDING RIMONABANT

We also showed successful results in reversing metabolic syndrome in obese patients with MetS receiving combination of fenofibrate and the recently withdrawn rimonabant^[28]. The combination treatment resulted in a significantly more pronounced reduction in the number of metabolic syndrome criteria compared with fenofibrate monotherapy ($P < 0.05$)^[28].

CONCLUSION

Taken together, these data suggest that combination treatment that includes a weight loss drug helps to improve lipoprotein profile, carbohydrate metabolism variables, hypertension and many other CVD risk factors or markers. Furthermore, this combination treatment reduced the presence of MetS criteria in obese patients with MetS.

These results are promising for patients with obesity and MetS. These patients need a multifactorial treatment targeting excess body weight, hyperlipidaemia and hypertension to reduce CVD risk factors. Though the population size was small, promising results from these findings indicate a need for double-blind, randomized trials examining the effects of such multifactorial treatment in hard CVD endpoints in patients with T2DM.

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Diabetic retinopathy: Role of inflammation and potential therapies for anti-inflammation

Gregory I Liou

Gregory I Liou, Department of Ophthalmology, Medical College of Georgia, GA 30912, United States

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Correspondence to: Gregory I Liou, PhD, Professor, Department of Ophthalmology, Medical College of Georgia, 1120 15th Street, Augusta, GA 30912, United States. giliou@mcc.edu
 Telephone: +1-706-7214599

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Abstract

Diabetic retinopathy is a leading cause of blindness among working-age adults. Despite many years of research, treatment options for diabetic retinopathy remain limited and with adverse effects. Discovery of new molecular entities with adequate clinical activity for diabetic retinopathy remains one of the key research priorities in ophthalmology. This review is focused on the therapeutic effects of cannabidiol (CBD), a non-psychoactive native cannabinoid, as an emerging and novel therapeutic modality in ophthalmology based on systematic studies in animal models of inflammatory retinal diseases including diabetic retinopathy - a retinal disease associated with vascular-neuroinflammation. Special emphasis is placed on novel mechanisms which may shed light on the pharmacological activity associated with CBD preclinically. These include a self-defence system against inflammation and neurodegeneration mediated by inhibition of equilibrative nucleoside transporter and activation of adenosine receptor by treatment with CBD.

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Key words: Cannabidiol; Anti-inflammation; Diabetic retinopathy; Retinal microglia; Adenosine receptors; Equilibrative nucleoside transporters

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INTRODUCTION

During the past decade, it has become clear that inflammation is a key feature in diabetes that leads to long-term complications in specific organs, in particular the eye and kidney. In the eye, the major complication is diabetic retinopathy, a leading cause of blindness in the Western world affecting three-fourths of diabetic patients within 15 years after onset of the disease^[1,2]. Many diabetic patients are referred to an ophthalmologist for evaluation and treatment only after visual complications have already occurred. The recommended treatment for diabetic retinopathy has been laser photo-coagulation but the procedure also destroys neural tissues. Therefore, there is a great need for the development of new non-invasive therapies. These visual complications are most likely associated with oxidative stress and inflammation. Our research in diabetic retinopathy has focused on delineating the inflammatory and neurodegenerative processes involved. We have identified new non-invasive receptor-based therapies for mitigating the retinal damage associated with diabetes. This review is focused on the therapeutic effects of cannabidiol (CBD) on animal models of diabetic retinopathy. Special emphasis is placed on novel mechanisms described in recent studies of retinal models which help to explain some of the pharmacological effects observed with CBD.

DIABETIC RETINOPATHY (DR)

DR is a chronic ocular disorder that, if untreated, will

lead to legal blindness. In the United States, over 20 million adults (or 10% of the total population) currently have diabetes. Of this group, over 12 000 patients will be diagnosed with new-onset blindness annually, making it one of the leading causes of legal blindness in Americans within the age group of 20-74^[3]. Type I diabetics usually have high incidence of retinopathy although retinopathy occurs in almost all patients with diabetes for 20 years or more^[1]. The earliest detectable signs of retinopathy are categorized as nonproliferative diabetic retinopathy (NPDR). NPDR is clinically subdivided into mild, moderate and severe categories. Loss of retinal pericytes and alterations in retinal blood flow are preclinical changes that are often non-detectable by physical exam^[4,5]. Retinal venous dilation and microaneurysms are the first alterations detectable by ophthalmoscopy. Following these alterations, intraretinal hemorrhage and exudation may occur. These may then lead to macular edema, which, if untreated may lead to irreversible vision loss and blindness. As hyperglycemia persists, the disease progresses to moderate and severe NPDR which presents with hemorrhages and venous beading, suggesting decreased retinal circulation and dilated capillaries^[6].

Proliferative diabetic retinopathy (PDR) is the next stage when proliferation of new blood vessels begins. Approximately 50 percent of patients with severe NPDR progress to PDR within one year^[7]. This stage is characterized by the onset of ischemia-induced new vessel proliferation from the optic nerve head as well as in the retina. These new vessels are fragile and tend to bleed easily resulting in vitreous hemorrhage. If untreated, the neovascularization will undergo fibrosis and contraction leading to traction retinal detachments. Additional complications may include neovascular glaucoma due to sprouting of new vessels on the iris and in the trabecular meshwork of the anterior chamber^[8].

DR is a vascular-neuroinflammatory disease

The early signs of diabetic retinopathy in experimental diabetic models include vascular inflammatory reactions due to oxidative stress, pro-inflammatory cytokines, and the consequent binding of leukocyte adhesion molecules CD18 and intercellular adhesion molecule 1 (ICAM-1)^[9]. These reactions lead to breakdown of the blood-retinal barrier (BRB) function, vascular occlusion and tissue ischemia, which in turn leads to neuronal cell death^[9-14]. However, diabetes could also directly affect metabolism within the neural retina leading to neuronal cell death. Whether diabetes affects vascular or neural retina first, both microglial and macroglial cells are activated^[15]. The function of activated macroglia in transporting^[16] and metabolizing glutamate may be impaired^[17] (unpublished observations). This leads to glutamate accumulation^[18-20]. Glutamate excitotoxicity occurs *via* activation of N-methyl-D-aspartic acid (NMDA) and non-NMDA receptors, to directly or indirectly induce calcium influx and the release of superoxides, leading to neuronal cell death^[21]. This is followed by neuro-inflammation, during which

activated microglial cells migrate toward dying neurons and release inflammatory cytokines to further exacerbate the damage^[22]. These findings suggest that pharmacological interventions that reduce oxidative stress and inflammation might be effective neuroprotectants for diabetic retinopathy^[20,23].

Microglia in DR

Normally quiescent microglia become activated during early diabetes^[24-27]. Cytokines such as interleukin (IL)-1 β , IL-6, γ -interferon, and tumor necrosis factor- α (TNF- α) have been shown to directly activate microglia^[28,29]. Activated microglia release (or promote the release of) glutamate, reactive oxygen species (ROS), IL-1 β , IL-3, IL-6, TNF- α , vascular endothelial growth factor (VEGF), lymphotoxin, matrix metalloproteinases (MMPs) and nitric oxide (NO)^[15,30]. The cytokines IL-1 β , IL-6, TNF- α , and lymphotoxin alter expression of vascular cell adhesion molecules to recruit lymphocytes and macrophages to injury sites^[31]. Lymphotoxin, TNF- α , NO and ROS can directly kill cells^[32,33]. VEGF, NO and MMPs can weaken the BRB, thus enhancing the infiltration of leukocytes into the retina. It remains unclear why diabetes would incite microglia activation in the retina but research on retinal microglia activation may provide substantial insights into the pathogenesis of DR^[34]. Cultured microglia have been used extensively to study microglial behavior. Treatment of microglia or macrophage-like cells with advanced glycation end-products (AGE) or Amadori-albumin^[35,36], high glucose^[37] or with endotoxins such as lipopolysaccharide (LPS) has been used as a model to simulate inflammation^[38-40].

ROLES OF ADENOSINE RECEPTORS (ARs) AND NUCLEOSIDE TRANSPORTERS IN INFLAMMATION

Adenosine, an endogenous purine nucleoside, has been proposed to modulate a variety of physiological responses by stimulating specific extracellular receptors^[41-43]. ARs have been classified as A₁, A_{2A}, A_{2B}, and A₃ receptors^[44]. Under stress and ischemia conditions, the local tissue concentrations of extracellular adenosine are increased due to the release of adenosine itself, or of AMP, which is metabolized extracellularly to adenosine. This increased adenosine can protect against excessive cellular damage via a negative feedback mechanism^[45] (unpublished observations). Adenosine released at inflamed sites exhibits anti-inflammatory effects through A_{2A}AR^[46]. Sub-threshold doses of an inflammatory stimulus that caused minimal tissue damage in wild-type mice were sufficient to induce extensive tissue damage and more prolonged and higher levels of pro-inflammatory cytokines in knock-out mice that lacked the A_{2A}AR (A_{2A}AR $-/-$ mice)^[47]. A_{2A}AR agonist treatment blocked the inflammation, functional and histological changes associated with diabetic nephropathy in wild-type diabetic mice, whereas it had no

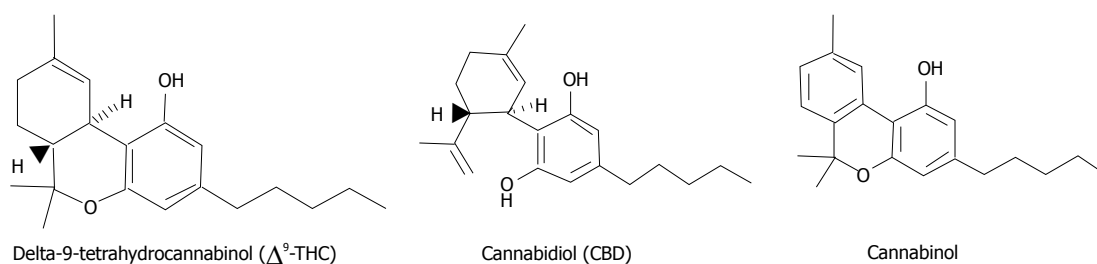


Figure 1 The best-known cannabinoids from marijuana. (-)-Δ⁹-tetrahydrocannabinol (THC), but not cannabinol or (-)-cannabidiol (CBD), is known to exert psychotropic effects.

effect on the A_{2A}AR -/- diabetic mice^[48]. A_{2A}AR, a Gs-protein-coupled receptor, can increase levels of immunosuppressive cAMP in microglia or other immune cells^[49]. Stimulation of the A_{2A}AR decreases leukocyte adhesion and blocks the associated release of oxygen free radicals^[50]. Adenosine released can activate endothelial adenosine receptors, leading to increases in intracellular cAMP and resealing of the endothelial junctions thereby promoting vascular barrier function^[51]. Moreover, A_{2A}AR activation induces the synthesis and release of nerve growth factor thereby is neuroprotective^[52].

Although adenosine and its agonists are protective in animal models of inflammation, their therapeutic application has been limited by systemic side effects such as hypotension, bradycardia, and sedation^[53]. Moreover, adenosine usually disappears very rapidly in physiological or inflammatory conditions due to rapid reuptake and subsequent intracellular metabolism^[54]. Endogenous adenosine levels at inflamed sites are reported to increase further because of the increased need for energy supplied by ATP, which is metabolized to AMP and adenosine ultimately^[55]. In addition, the activity of 5'-nucleotidase, which metabolizes AMP to adenosine, is reported to increase in inflammatory conditions^[56]. It is therefore assumed that prevention of adenosine uptake into the cells and its subsequent metabolism can selectively enhance extracellular concentrations of adenosine at inflamed sites, resulting in an anti-inflammatory effect^[57]. Protective or ameliorating effects of adenosine uptake inhibitors in ischemic cardiac and cerebral injury, organ transplantation, seizures, thrombosis, insomnia, pain and inflammatory diseases have been reported^[58]. Preclinical and clinical results indicate the possibility of therapeutic application of adenosine uptake inhibitors^[58,59].

Adenosine reuptake and degradation

Adenosine disappears rapidly in physiological or inflammatory conditions due to rapid reuptake *via* nucleoside transporters (NTs) and subsequent intracellular metabolism^[54]. There are two subtypes of NTs: Concentrative NTs which are dependent on the presence of extracellular sodium, and equilibrative NT (ENTs). In the microglial cells, the majority of adenosine transport is not affected by sodium removal suggesting ENTs are the primary transporters functioning in these cells^[60]. ENTs are further classified into two subtypes on the

basis of their sensitivities to inhibition by the drug S-(4-nitrobenzyl)-6-thioinosine [nitrobenzylmercaptapurine riboside (NBMPR)]. NBMPR-sensitive ENTs bind NBMPR with high affinity and have the functional designation equilibrative sensitive (ENT1). NBMPR-insensitive transporters are designated ENT2. Dipyridamole, an inhibitor for both ENT1 and ENT2^[61], is used clinically as a coronary vasodilator and a platelet aggregation inhibitor^[62,63]. Dipyridamole plus aspirin improves retinal vasculature patterns in experimental diabetes^[64].

Role of ENT1 in adenosine function in diabetes

ENT1 plays an integral role in adenosine function in diabetes by regulating adenosine levels in the vicinity of adenosine receptors. It was reported that adenosine uptake by ENT1 in human aortic smooth muscle cells (HASMCs) was increased by hyperglycemia^[65]. To provide insight into mechanisms by which ENT1 was modulated by hyperglycemia, kinetic studies of adenosine transport and [³H]NBMPR binding were performed^[65]. The results show that *V*_{max} (representing the number of ENT1) of adenosine transport in high glucose (HG)-treated HASMCs was increased without affecting *K*_m (representing the affinity of ENT1). Similarly, *B*_{max} (representing the number of ENT1) of the high-affinity [³H]NBMPR binding was increased without affecting *K*_d (representing the affinity of ENT1). Consistent with these observations, HG increased mRNA and protein expression of ENT1. Pathologically, the increase in ENT1 activity in diabetes may affect the availability of adenosine in the vicinity of adenosine receptors and, thus, alter vascular functions in diabetes. Pharmacological intervention of ENT1 activity may prove to be effective therapeutics in diabetes. Current studies are in progress to elucidate the effect of hyperglycemia on the function and expression of ENT1 in the retinal microglial and vascular endothelial cells.

CANNABINOID AND CANNABINOID RECEPTORS

The best-known cannabinoids from marijuana are (-)-Δ⁹-tetrahydrocannabinol (THC), cannabinol (CBN), and (-)-cannabidiol (CBD) (Figure 1)^[66]. THC, but not CBN or CBD, is known to exert psychotropic effects^[67,68]. Cannabinoids are also known to be therapeutic with

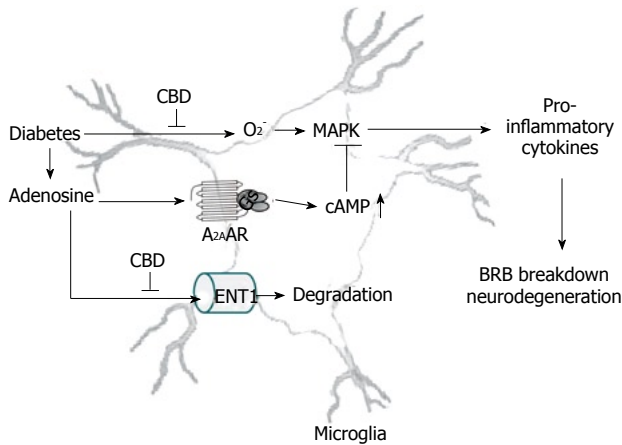


Figure 2 The hypothetical mechanism of anti-inflammation effect CBD in diabetic retinopathy. Diabetes causes release of adenosine and pro-inflammatory cytokines via superoxide formation and MAPK activation, leading to DR. Adenosine-initiated anti-inflammation via A_{2A}AR-Gs-cAMP signaling is terminated rapidly due to adenosine reuptake by equilibrative nucleoside transporter (ENT) and subsequent metabolism. CBD blocks superoxide formation and inhibits adenosine reuptake via inhibiting ENT1, thereby activating A_{2A}AR-Gs-cAMP signaling.

properties of anti-inflammation^[69,70] and anti-oxidation^[71]. Cannabinoids produce their biological effects by acting through at least two receptors. Receptor CB1 (cloned) is responsible for psychoactivity and is expressed in the brain^[72] and retinal neurons^[73,74]. Receptor CB2 (cloned) is expressed in immune cells^[75] and cerebral microglial cells^[76], but also in the retina^[77]. These receptors are coupled to Gi/o proteins to inhibit adenylyl cyclase activity and immediate early gene signaling pathway(s)^[78]. Receptor CB1 is also coupled through Gi/o proteins to inhibit voltage-sensitive calcium channels^[79] and activate potassium channels^[80].

CBD has very low affinity to either CB1 or CB2^[81,82]. This low affinity of CBD for CB1 accounts for its inability to produce the subjective “high” and cognitive effects that are characteristic of marijuana and THC. CBD is very effective as a scavenger of ROS. The antioxidative effect of CBD is superior to α -tocopherol and ascorbate *in vitro* and *in vivo*^[71] due to its ability to scavenge ROS and block NADPH oxidase^[40]. CBD also has potent anti-inflammatory actions and have been shown to decrease inflammatory cytokines in arthritis^[83] and in diabetes^[12], prevent cerebral damage during ischemia^[84] and to prevent cerebral infarction^[85]. CBD is well tolerated when chronically administered to humans^[86] and has been approved for the treatment of inflammation, pain and spasticity associated with multiple sclerosis in patients since 2005^[87]. CBD attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption in human coronary endothelial cells^[88]. It also decreases the incidence of diabetes in non-obese diabetic mice^[89] and is neuroprotective and BRB-preserving in streptozotocin-induced diabetes^[12]. Most recently, CBD has been shown to decrease retinal inflammation by blocking ROS and TNF- α formation, p38 MAP kinase

activation and microglial activation^[40]. Current data in the effects of intraocularly introduced CBD in diabetic animal model are consistent with its anti-inflammatory activity (unpublished observations).

CBD enhances AR-mediated anti-inflammation

It has recently been shown that nanomolar concentrations of CBD or THC could inhibit uptake of adenosine by ENT1 in murine microglia, RAW264.7 macrophages^[60] and in rat retinal microglia^[39]. CBD synergistically enhances adenosine's TNF- α suppression upon LPS treatment. Moreover, *in vivo* treatment with a low dose of CBD decreases TNF- α production in serum in the LPS-treated mice; this effect is reversed by treatment with an A_{2A}AR antagonist and abolished in A_{2A}AR -/- mice^[60]. Similar results are observed in the rat retina^[39]. These studies demonstrate that CBD has the ability to enhance adenosine signaling through inhibition of uptake and provide a non-cannabinoid receptor mechanism by which CBD can decrease endotoxin-induced inflammation. Current data suggest that CBD inhibits diabetes-induced retinal inflammation by the same mechanism (unpublished observations). A hypothetical pathway illustrating how CBD works to reduce retinal inflammation in diabetes is shown in Figure 2.

CONCLUSION

Recent evidence suggests that local inflammation plays a major role in the pathogenesis of diabetic retinopathy. The function of CBD as an antioxidant to block oxidative stress and as an inhibitor of adenosine reuptake to enhance a self-defense mechanism against retinal inflammation represents a novel therapeutic approach to the treatment of ophthalmic complications associated with diabetes. This study is important for the development of adenosine reuptake inhibitors as a potentially novel and effective therapy for diabetic retinopathy. However, the therapeutic values of these agents should be confirmed by clinical trials. Furthermore, depending on the difference in the genetic make-ups for the metabolism and pharmacological target of CBD, it may be important to consider CBD as a personalized medicine, i.e. adjusted dosages according to individual's genetic make-ups, to offer significant advantages over traditional clinical approaches^[90].

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Effect of vildagliptin as add-on therapy to a low-dose metformin

Claudia Filozof, Sherwyn Schwartz, James E Foley

Claudia Filozof, Diabetes Principal Medical Scientific Expert, Novartis Pharma AG, Fabrikstrasse 4-4.43.4, CH-4056 Basel, Switzerland

Sherwyn Schwartz, Diabetes and Glandular Disease Clinic, San Antonio, TX 78229, United States

James E Foley, Novartis Pharmaceuticals Corporation, East Hanover, NJ 07936, United States

Author contributions: Filozof C and Foley JE wrote this paper on behalf of many individuals at Novartis who contributed to the design, implementation, analysis and reporting of the data including Valentin M, Colin L, Holmes D, Thuren T, Buccheit F, Degen J and Kothny W; Schwartz S wrote this paper as the principal investigator on behalf of the study investigators; Filozof C and Foley J are employees of Novartis Pharmaceuticals Corporation and hold stocks in the company; Schwartz S was involved in the conduct of the study as a clinical trial investigator. No additional known conflict of interest exists and no honoraria were offered or received for coauthor participation in the writing of this manuscript.

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Correspondence to: Claudia Filozof, MD, PhD, Diabetes Principal Medical Scientific Expert, Novartis Pharma AG, Fabrikstrasse 4-4.43.4, CH-4056 Basel,

Switzerland. claudia.filozof@novartis.com

Telephone: +41-61-3242987 Fax: +41-61-3247921

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Abstract

AIM: To evaluate the efficacy and safety of the addition of vildagliptin to low-dose metformin and compare it to an uptitration of metformin in type 2 diabetes mellitus (T2DM) patients who have inadequate control with metformin monotherapy.

METHODS: Eligible patients were randomized to receive vildagliptin 100 mg qd or metformin (500 mg qd for 2 wk and then 500 mg bid) added to open label metformin 500 mg bid for the 24 wk. The primary endpoint was baseline to endpoint hemoglobin A_{1c} (HbA_{1c}) change.

RESULTS: The adjusted mean change from baseline in HbA_{1c} at the 24th wk was -0.51% in the vildagliptin/metformin group (mean baseline HbA_{1c}: 7.4%) and -0.37% in the metformin monotherapy group (mean baseline HbA_{1c}: 7.3%). The mean difference was -0.14% with 95% Confidence Interval (-0.24%, -0.05%). As non-inferiority (margin of 0.4%) was achieved, a test for superiority was performed. This test showed statistically significant superiority of the combination over monotherapy group ($P = 0.002$). Gastrointestinal (GI) adverse events were significantly more frequent in the metformin group than the combination group (21.0% vs 15.4%, $P = 0.032$).

CONCLUSION: In patients with T2DM inadequately controlled with metformin up to 1000 mg daily, the addition of vildagliptin 100 mg daily achieved larger HbA_{1c} reduction with fewer GI events than with increasing the metformin dose.

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Key words: Vildagliptin; Metformin; Dipeptidyl peptidase-4; Hemoglobin A_{1c}; Glucagon-like peptide-1; Gastrointestinal side effects

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INTRODUCTION

Metformin is the gold standard first-line treatment for type 2 diabetes mellitus (T2DM)^[1]. This recommendation is based on its therapeutic efficacy, a relatively low

incidence of hypoglycemia and no weight gain. In addition, data from the UK Prospective Diabetes Study showed that metformin can improve cardiovascular outcomes in overweight patients with T2DM^[2]. However, gastrointestinal (GI) symptoms such as nausea, diarrhea and abdominal pain are common and often lead to discontinuation. The incidence of GI disturbances has been reported to be dose-related and may remit if the dose is reduced^[3].

Vildagliptin is a potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor that improves pancreatic islet function as evidenced from the improved ability of the α -cell and β -cell to sense and respond to glucose following treatment^[4]. In addition, vildagliptin inhibits hepatic glucose production during meals as well as during the overnight post-absorptive period^[5]. Furthermore, vildagliptin has been shown to improve insulin resistance^[6]. Targeting multiple defects using agents with synergistic or complementary mechanisms of action may be beneficial in achieving glycemic targets. Mechanistic studies have suggested that vildagliptin may be particularly effective when used in combination with metformin with a potential synergistic effect. The effect of vildagliptin to increase plasma levels of intact glucagon-like peptide-1 (GLP-1) was enhanced in patients receiving concomitant metformin while there was no evidence of an effect of metformin on plasma DPP-4 activity or on GLP-1 or GIP in the absence of vildagliptin^[7]. When glucose levels are above normal fasting levels, enhanced GLP-1 levels stimulate insulin secretion and inhibit glucagon secretion^[8,9]. In addition, enhanced GLP-1 levels may increase islet cell mass. In prior clinical studies, vildagliptin added to ongoing metformin monotherapy significantly improved fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA_{1c}). These effects were associated with an improvement in measures of β -cell function, no weight gain and no increase in the incidence of hypoglycemia^[10,11].

The present study was designed to compare the glycemic efficacy and safety of the addition of vildagliptin (100 mg qd) to low-dose metformin (500 mg bid) with that of an upward titration of metformin in patients with T2DM with inadequate glycemic control on metformin monotherapy.

MATERIALS AND METHODS

Study population

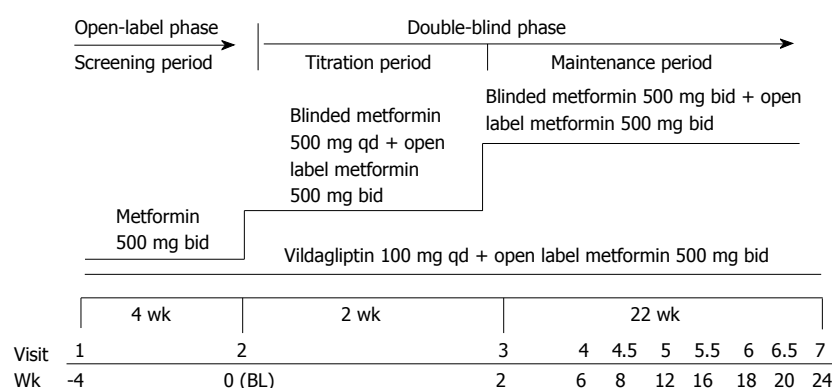
Male and female (non-fertile or using a medically approved birth control method) patients aged 18-78 years with HbA_{1c} 6.5%-9.0%, FPG < 270 mg/dL (15 mmol/L) and a body mass index (BMI) of 22-45 kg/m² who received metformin 850-1000 mg daily for at least 2 mo prior to screening were eligible to participate in the study.

Patients were excluded if they had a history of type 1 or secondary forms of diabetes, evidence of significant diabetic complications, acute infections, myocardial infarction, unstable angina or coronary artery bypass surgery within the previous 6 mo. Congestive heart failure

requiring pharmacological treatment, malignancy (not including basal cell skin cancer) and liver disease, such as cirrhosis or chronic active hepatitis, also precluded participation. Patients with electrocardiogram (ECG) abnormalities, such as Torsades de pointes, sustained and clinically relevant ventricular tachycardia or ventricular fibrillation, second-degree atrioventricular (AV) block (Mobitz 1 and 2), third-degree AV block, and prolonged QTc (> 500 ms) were also excluded. Patients with any of the following laboratory abnormalities were also excluded: Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) greater than two times the upper limit of the normal range at visit 1, confirmed by repeat measurement within 3 working days; total bilirubin greater than two times the upper limit of the normal range and/or direct bilirubin greater than the upper limit of the normal range at visit 1, confirmed by repeat measurement within 3 working days; clinically significant renal dysfunction as indicated by serum creatinine levels ≥ 1.5 mg/dL (132 μ mol/L) in males, ≥ 1.4 mg/dL (123 μ mol/L) in females, or a history of abnormal creatinine clearance; clinically significant TSH values outside normal range at visit 1; fasting triglycerides > 700 mg/dL (7.9 mmol/L) at visit 1. Patients were also excluded if they were taking any of the following medications/treatments: chronic insulin treatment (> 4 wk of treatment in the absence of an intercurrent illness) within the past 6 mo and/or any oral antidiabetic drug other than metformin within 3 mo prior to visit 1; chronic oral or parenteral corticosteroid treatment (> 7 consecutive days of treatment) within 8 wk prior to visit 1; treatment with growth hormone or similar drugs; treatment with class I a, I b and I c or III antiarrhythmics; treatment with any drug with a known and frequent toxicity to a major organ system within the past 3 mo (i.e. cytostatic drugs). Finally, contraindications and warnings according to the country-specific label for metformin, history of active substance abuse (including alcohol) within the past 2 years and participation in previous vildagliptin studies also precluded participation.

A patient's treatment was discontinued if one or more of the following pertained: unsatisfactory therapeutic effect [defined as FPG > 240 mg/dL (13.3 mmol/L) after 12 wk of treatment confirmed by a repeated measurement in the absence of an intercurrent illness]; symptoms of worsening hyperglycemia in the absence of any intercurrent illness or other incidental circumstances potentially causing deterioration of glucose control; the occurrence of an adverse event (AE) including GI side effects or clinically significant laboratory change or an abnormality that, in the judgment of the investigator, warranted discontinuation of the treatment; pregnancy; severe or frequent hypoglycemia (i.e. unexplained hypoglycemic events requiring the assistance of another person to treat or > 3 hypoglycemic events per wk); treatment with prohibited concomitant medications.

All patients provided written informed consent to participate and the study protocol was reviewed and



approved by the appropriate committees and authorities for each study site. The study was performed in accordance with the Declaration of Helsinki.

Study design

The overall study design of this 24-wk, randomized, double-blind trial is presented in Figure 1. Each patient attended a screening visit (wk 4) to assess the inclusion/exclusion criteria. All patients received open-label metformin 500 mg bid at visit 1 for a period of 4 wk. Eligible patients were then randomized to receive either vildagliptin 100 mg qd or metformin 500 mg qd (double-dummy design) for 2 wk and then metformin 500 mg bid. All patients continued with the open-label metformin 500 mg bid for the 24 wk. Dose adjustments of vildagliptin or open-label metformin were not allowed at any time after randomization.

If the patients were unable to tolerate the study drug due to GI symptoms following the uptitration, metformin could be reduced by one tablet only until the GI symptoms had improved. The dose had to be then restored gradually over 1-2 wk based on the patient's ability to tolerate the study drug. No rescue medication (additional oral antidiabetic drugs or insulin to control glycemia) was permitted in this study; patients with unsatisfactory therapeutic effect were discontinued from the study. Patients who were prematurely withdrawn from the study were not replaced. Efficacy and tolerability were assessed in eight visits over the 24 wk.

Study assessments

The primary efficacy assessment was change in HbA_{1c} from baseline. Secondary assessments included FPG, body weight and GI tolerability.

HbA_{1c}, FPG, body weight and vital signs were assessed at screening, baseline and wk 2, 6, 12, 18 and 24. Liver function (AST, ALT, direct and total bilirubin and alkaline phosphatase) was monitored by taking blood samples at wk 2, 6, 8, 16 and 20. Standard hematology and biochemistry laboratory assessments were made at screening, baseline and wk 12 and 24. Fasting lipid levels [triglyceride, total/calculated low-density lipoprotein, high-density lipoprotein (HDL), non-HDL cholesterol and calculated very-low-density lipoprotein cholesterol] were measured at baseline and at the end of the study.

ECG was performed at screening and at wk 0 and 24. All AEs were recorded. Patients were provided with glucose-monitoring devices and supplies and instructed on their use. Patients were educated on hypoglycemic symptoms and their treatment.

All laboratory assessments were made by a central laboratory (Covance-US, Indianapolis, IN, USA). HbA_{1c} was measured with an ion exchange high-performance liquid chromatography method and all assays were performed with standardized and validated procedures according to Good Laboratory Practice.

An independent Cardiovascular and Cerebrovascular Adjudication Committee reviewed all occurrences of selected cardiovascular AEs, while an independent Internal Medicine Adjudication Committee reviewed occurrence of selected GI disorders (GI hemorrhage), general system disorders (generalized edema/anasarca), renal failure, skin and subcutaneous tissue disorders (angio-edema, generalized urticaria), and deaths (non-cardiovascular or cerebrovascular cause).

Statistical analyses

The primary efficacy analysis assessed whether (margin of 0.4%) the study treatments were non-inferior with regard to the HbA_{1c} at wk 24 or at the final visit (for patients who did not have HbA_{1c} measurement at wk 24, the last observation carried forward approach was adopted). An analysis of covariance (ANCOVA) model was fitted including terms for treatment and baseline HbA_{1c} as the covariate. When non-inferiority was achieved, a test for superiority was performed. The analysis of the primary efficacy variable using the intention to treat (ITT) population (received at least one dose of each study drug and had at least one post-baseline HbA_{1c} assessment) was the primary basis of conclusion. An analysis based on the per protocol (PP) population was also performed to assess the robustness of the conclusion. The per protocol population included ITT patients who completed at least 22 wk of treatment and those who discontinued the study due to unsatisfactory therapeutic effect (FPG > 240 mg/dL) after 12 wk of treatment, provided they had no major protocol deviations and had a valid assessment of HbA_{1c} within 7 d after the last dose of study drug. The change from baseline in FPG and body weight at the end of the study was analyzed using the ANCOVA model at the statistical significance level of 0.05.

Table 1 Demographic and baseline characteristics (randomized population)

Demographic variable	Vilda 100 mg qd/metformin 500 mg bid <i>N</i> = 456	Metformin up to 1000 mg bid <i>N</i> = 458	Total <i>N</i> = 914
Gender; female, <i>n</i> (%)	226 (49.6)	252 (55)	478 (52.3)
Age; mean ± SD (years)	56.9 ± 9.76	57.0 ± 10.02	57.0 ± 9.89
Age group (≥ 65 years), <i>n</i> (%)	106 (23.2%)	114 (24.9%)	220 (24.1%)
Race			
Caucasian	242 (53.1%)	237 (51.7%)	479 (52.4%)
Asian (non-indian subcontinent)	44 (9.6%)	44 (9.6%)	88 (9.6%)
Hispanic or latino	147 (32.2%)	145 (31.7%)	292 (31.9%)
Black	9 (2.0%)	12 (2.6%)	21 (2.3%)
Asian (indian subcontinent)	3 (0.7%)	5 (1.1%)	8 (0.9%)
Native american	1 (0.2%)	2 (0.4%)	3 (0.3%)
Other	10 (2.2%)	13 (2.8%)	23 (2.5%)
Body weight; mean ± SD (kg)	84.6 ± 17.01	84.4 ± 18.94	84.5 ± 17.99
BMI (kg/m ²); mean ± SD	31.1 ± 5.11	31.2 ± 5.47	31.1 ± 5.29
HbA _{1c} (%); mean ± SD	7.4 ± 0.78	7.3 ± 0.79 ^a	7.3 ± 0.79
FPG (mmol/L); mean ± SD	8.7 ± 2.28	8.5 ± 2.25	8.6 ± 2.27
Duration of type 2 diabetes; mean ± SD (years)	4.6 ± 4.91	4.7 ± 4.94	4.7 ± 4.92

Demographic information is collected on the day of the screening measurement (wk-4, Visit 1). ^aHemoglobin A_{1c} (HbA_{1c}) measurements at Visit 1 not available for one patient (*n* = 457). FPG: Fasting plasma glucose; BMI: Body mass index.

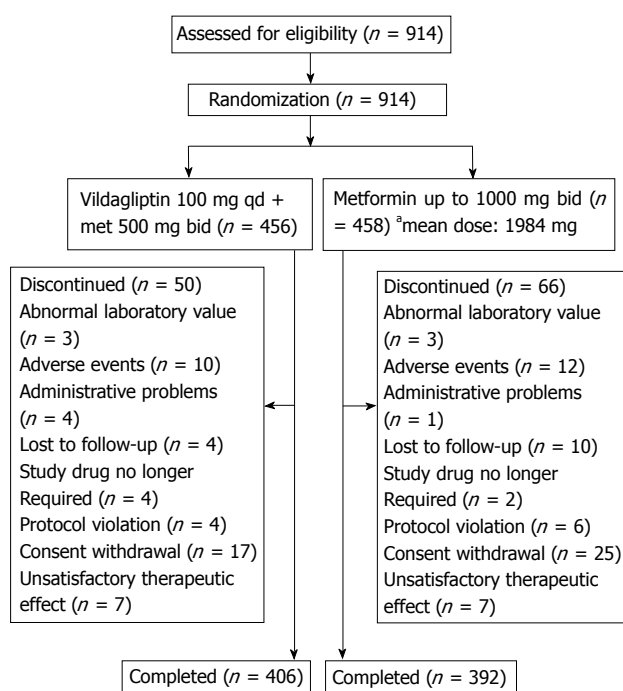


Figure 2 Patient flow diagram. ^aDose after titration at Visit 4 (wk 6).

The assessment of safety was based mainly on the frequency of treatment-emergent AEs, on the number of post-baseline laboratory values that fell outside pre-determined ranges and on the frequency and severity of hypoglycemic events. The incidence of patients with at least a single GI event was compared between treatment groups using a χ^2 -test.

RESULTS

A total of 914 patients (Figure 2) were randomized to

receive either vildagliptin 100 mg qd/metformin 500 mg bid (*n* = 456) or metformin monotherapy (*n* = 458 patients) up to 1000 mg bid (final mean metformin dose after uptitration at visit 4 was 1984 mg). Of these patients, 798 (87.3%) completed the study. Discontinuation was higher in patients from the metformin group (14.4%) than in the vildagliptin/metformin group (11.0%). The most frequent reason for discontinuation was withdrawal of consent which was more frequent in the metformin than in the vildagliptin/metformin group (5.5% *vs* 3.7%).

The patient baseline demographics were comparable between the treatment groups (Table 1). There was no imbalance observed in any concomitant therapies across both groups. The primary efficacy endpoint was the change from baseline to endpoint in HbA_{1c} in the ITT population. The adjusted mean change from baseline in HbA_{1c} at 24 wk was -0.51% in the vildagliptin/metformin group (mean baseline HbA_{1c}: 7.4%), and -0.37% in the metformin monotherapy group (mean baseline HbA_{1c}: 7.3%). The mean difference was -0.14% with 95% Confidence Interval (CI) (-0.24%, -0.05%). As non-inferiority (margin of 0.4%) was achieved, a test for superiority was performed. This test showed statistically significant superiority of the combination over monotherapy group (*P* = 0.002). Consistent results were observed in the PP population.

Mean changes in HbA_{1c} (%) over time are depicted in Figure 3. Starting with similar baseline values, HbA_{1c} in the vildagliptin/metformin group was consistently lower than in the metformin group at all study visits. For all baseline HbA_{1c} pre-defined categories, changes were larger in the vildagliptin/metformin than in the monotherapy group. In both treatment groups, patients with a higher baseline value (HbA_{1c} > 8%) showed the greatest reduction in HbA_{1c} from baseline at endpoint. In patients with HbA_{1c} > 8% at baseline, the mean

Table 2 Number (%) of patients reporting common AEs ($\geq 2\%$ in any group) and biochemistry abnormalities (safety population)

Preferred term	Vilda 100 mg qd/metformin 500 mg bid <i>N</i> = 456 <i>n</i> (%)	Metformin up to 1000 mg bid <i>N</i> = 458 <i>n</i> (%)
Any preferred term	220 (48.2)	237 (51.7)
Diarrhea	21 (4.6)	39 (8.5)
Headache	18 (3.9)	28 (6.1)
Nasopharyngitis	15 (3.3)	13 (2.8)
Back pain	14 (3.1)	18 (3.9)
Dizziness	14 (3.1)	16 (3.5)
Flatulence	13 (2.9)	8 (1.7)
Upper respiratory tract infection	13 (2.9)	10 (2.2)
Arthralgia	11 (2.4)	6 (1.3)
Hypertension	11 (2.4)	12 (2.6)
Nausea	11 (2.4)	22 (4.8)
Pharyngitis	10 (2.2)	12 (2.6)
Pain in extremity	8 (1.8)	10 (2.2)
Urinary tract infection	8 (1.8)	24 (5.2)
Dyspepsia	7 (1.5)	11 (2.4)
Influenza	7 (1.5)	11 (2.4)
Abdominal pain	5 (1.1)	12 (2.6)
Laboratory evaluation		
Any notable abnormality ^a	14 (3.2)	14 (3.2)
Alkaline phosphatase $\geq 3 \times$ ULN	0 (0.0)	0 (0.0)
ALT (Alanine aminotransferase) $\geq 3 \times$ ULN	1 (0.2)	1 (0.2)
AST (aspartate aminotransferase) $\geq 3 \times$ ULN	1 (0.2)	2 (0.5)
Bilirubin (direct/conjugated) $\geq 3 \times$ ULN	0 (0.0)	0 (0.0)
Blood Urea Nitrogen (BUN) ≥ 9.99 mmol/L	9 (2.0)	6 (1.4) ^b
Creatine phosphokinase (CPK) $\geq 5 \times$ ULN	1 (0.2)	0 (0.0) ^b
Creatinine ≥ 176.8 μ mol/L	0 (0.0)	0 (0.0) ^b
Potassium ≤ 3.0 or ≥ 6.0 mmol/L	3 (0.7) ^c	5 (1.1) ^b
Sodium ≤ 125 or ≥ 160 mmol/L	1 (0.2)	0 (0.0) ^b

^aNo. of patients with evaluable criterion in both categories is 441; ^bNo. of patients with evaluable criterion is 437; ^cNo. of patients with evaluable criterion is 440; AEs: Adverse events; ULN: Upper limits of normal.

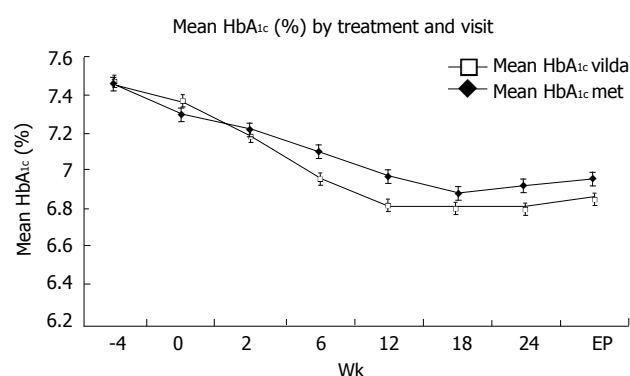


Figure 3 Mean HbA_{1c} (%) by treatment and visit. Data are presented as mean \pm SEM. vilda: vildagliptin/metformin; met: metformin.

reductions in HbA_{1c} from baseline to endpoint were $-0.65\% \pm 0.13\%$ and -0.51 ± 0.12 in the combination and monotherapy groups and $-0.46\% \pm 0.03\%$ and -0.31 ± 0.03 respectively in patients with HbA_{1c} $\leq 8\%$. In both treatment groups, patients with a BMI < 30 kg/m² at baseline showed a greater reduction (vildagliptin/metformin $-0.51\% \pm 0.05\%$; metformin monotherapy $-0.41\% \pm 0.05\%$) in HbA_{1c} compared to the changes in the more obese patients (vildagliptin/metformin -0.49 ± 0.05 ; metformin monotherapy -0.29 ± 0.05). HbA_{1c} changes were similar in older patients (≥ 65 years) and patients < 65 years of age.

The proportion of patients reaching HbA_{1c} $< 7\%$ was 49.5% in the vildagliptin/metformin group versus 43.5% in the metformin monotherapy group but the difference did not reach statistical significance ($P = 0.154$). The proportion of patients achieving HbA_{1c} $\leq 6.5\%$ was significantly higher in the combination group compared with the metformin monotherapy group (53.8% *vs* 41.2%).

The adjusted mean change from baseline in FPG was numerically higher in the vildagliptin/metformin than in the metformin group [-0.77 mmol/L and -0.59 mmol/L respectively; 95% CI for difference in the mean change between treatments ($-0.38, 0.02$), $P = 0.070$]. A reduction from baseline in body weight was observed at endpoint in both treatment groups. The change was significantly higher in the metformin group than in the combination group (-1.35 kg *vs* -0.62 kg, $P < 0.001$).

Safety and tolerability

The overall AE rate was comparable in patients receiving vildagliptin/metformin and metformin (48.2% and 51.7% respectively). The most frequently reported AEs (with an incidence of $\geq 2\%$) in both groups (Table 2) were diarrhea and headache. The incidence rates of AEs by primary system organ class were generally comparable between the two treatment groups except for GI AEs which were significantly more frequent in the metformin

monotherapy treatment group than in the vildagliptin group (21.0% *vs* 15.4%, $P = 0.032$).

The incidence of serious adverse events and AEs leading to discontinuation or causing study drug interruption were low and comparable between the treatment groups. Serious abdominal pain ($n = 2$) and diarrhea leading to discontinuation ($n = 2$) were reported in patients in the metformin group whereas no such cases were reported in the combination group.

The proportion of patients with clinically significant AEs confirmed by the Cardiovascular and Cerebrovascular Adjudication Committee were similar between both the treatment groups (1.3% and 1.1% in the combination and the metformin monotherapy respectively). Clinically significant AEs confirmed by the Internal Medicine Adjudication Committee were infrequent and were reported in three patients in the combination group (two patients with GI hemorrhage and one patient with renal failure) and one patient in the metformin monotherapy group reported angio-edema.

The incidence of edema (0.4% *vs* 0.4%), hepatic disorders (0.4% *vs* 0.4%), muscle-event (3.2% *vs* 4.6%) and paresthesia- (1.1% *vs* 1.1%), skin- (0.4% *vs* 0.2%) and vascular-related AEs (0.0% *vs* 0.2%) were similar or lower in the combination group compared with the metformin monotherapy group. One patient in each treatment group experienced one hypoglycemic event (grade 1). Both events were of mild severity and did not lead to discontinuation.

No major changes from baseline to endpoint were observed for any hematological, biochemical (Table 2), urinalysis parameter or vital signs. The frequency and nature of ECG changes from baseline to endpoint were comparable in the two treatment groups.

DISCUSSION

Previous studies have shown that addition of vildagliptin to patients inadequately treated with maximum tolerated doses of metformin leads to reductions in HbA_{1c} (~1%) relative to metformin alone^[7] and to equivalent reductions in HbA_{1c} (~1%) relative to metformin plus a TZD^[12]. Both of these studies were from baseline HbA_{1c} levels of ~8.5%. On the other hand, in a previous study, the combination of vildagliptin with low-dose metformin (1000 mg daily) achieved superior efficacy to high doses of metformin in patients with HbA_{1c} levels of ~8.7% without any associated GI tolerability issues^[13]. The evidence suggests, however, that earlier, more aggressive treatment is needed^[14]. The present study was designed to compare the benefits of more aggressive treatment by either escalating the dose of metformin or by adding vildagliptin to a lower dose of metformin in patients with lower baseline HbA_{1c} levels. The data showed that an addition of vildagliptin (100 mg daily) to a low-dose metformin (500mg bid) provided larger reductions in HbA_{1c} of 0.51% from a baseline HbA_{1c} of -7.3%-7.4% than reductions in HbA_{1c} of 0.37% seen in patients

where the metformin dose was increased to 2000 mg. Furthermore, a significantly higher proportion of patients achieved HbA_{1c} ≤ 6.5% (53.8% *vs* 41.2%) with no increase in the hypoglycemic event rate in the combination group compared with the metformin monotherapy group.

The present study also showed that GI events (diarrhea, nausea and abdominal pain) were less frequent in patients receiving vildagliptin added to low doses of metformin than in the patients treated with metformin monotherapy 2000 mg daily. This is important since, in clinical practice, metformin is uptitrated up to 2-3 g daily with many patients having GI AEs, which may lead to low compliance and treatment discontinuation. A dose-related incidence of GI adverse effects with metformin has been reported by some^[3] although not all studies^[15]. It has been suggested that the use of gradual dose escalation might explain the lack of a linear dose relationship for GI AEs^[15]. In any case, in the present study, even though metformin was increased gradually and the mean incidence of total GI disturbances was lower than the one reported in previous studies^[15], the incidences of diarrhea and nausea were twice as high in the metformin monotherapy group compared with the vildagliptin/metformin group (8.5 *vs* 4.5% and 4.8 *vs* 2.4% respectively). Although the total numbers of patients who discontinued due to adverse events was low in this 24-wk study, serious abdominal pain and diarrhea led four patients to discontinuation in the metformin monotherapy group but only one patient from the combination treatment group reported discontinuation.

The HbA_{1c} changes observed in this study with vildagliptin are consistent with previously reported data utilizing vildagliptin in patients with comparable glycemic control at baseline^[16]. Furthermore, the results are consistent with a similarly designed study in which the efficacy and safety of adding rosiglitazone to low dose of metformin was compared to increasing the dose of metformin in patients with T2DM^[17]. The rosiglitazone study with a comparable baseline glycemic control to the present vildagliptin study showed similar efficacy of the rosiglitazone/metformin combination group (mean change from baseline: -0.33%) compared with the uptitrated metformin monotherapy group (mean change from baseline: -0.13%). Similar to the present vildagliptin study, there were fewer episodes of diarrhea, abdominal pain or dyspepsia and the instances of withdrawal from the study due to GI disturbances were fewer in the combination group compared with the metformin group. In contrast, in this previous study, body weight increased by 1.79 kg after 24 wk of treatment in the metformin/rosiglitazone group relative to the high dose metformin group, whereas in the present study, a reduction from baseline in the body weight was observed at endpoint in both the treatment groups (-1.35 kg *vs* -0.62 kg, $P < 0.001$ in the metformin and metformin/vildagliptin group, respectively).

In summary, in patients with T2DM inadequately controlled with metformin up to 1000 mg daily, the addition

of vildagliptin 100 mg daily achieved larger HbA_{1c} reduction with fewer GI events. These data suggest that an early addition of vildagliptin to submaximal doses of metformin has the potential to offer benefits over up-titration of metformin with superior efficacy, lower incidence of GI AE, no increase in the hypoglycemic event rate and no weight gain.

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COMMENTS

Background

Metformin is an established gold standard and the first option for the treatment of type 2 diabetes. Previous clinical studies demonstrate that the addition of vildagliptin to metformin monotherapy in patients not reaching therapeutic targets HbA_{1c} \geq 8.5% with maximum tolerated doses of metformin significantly improved fasting plasma glucose and glycosylated hemoglobin by 1% with an efficacy similar to a TZD (with no weight gain). The evidence suggests, however, that earlier more aggressive treatment is needed. In clinical practice, metformin is up-titrated from 850 mg to 2-3 g or more before adding any second antidiabetic. High doses of metformin are associated with more Gastrointestinal (GI) events, potentially leading to lower compliance. The present study was designed to compare efficacy and safety of either adding vildagliptin to a low-dose of metformin (1g) or escalating the dose of metformin in patients with HbA_{1c} levels < between 6.5%-9%.

Research frontiers

In the present study, early add-on of vildagliptin to metformin, over up-titration of metformin doses achieve larger HbA_{1c} drops and lower incidence of GI adverse events with no increase in hypoglycemic events and no weight gain.

Innovations and breakthroughs

The evidence suggests that earlier more aggressive treatment with low risk of hypoglycemic events is needed to prevent long-term complications. This study showed that an early (HbA_{1c} 6.5%-9%) addition of vildagliptin to a low-dose metformin provided larger reductions in HbA_{1c} relative to increasing the metformin dose. Furthermore, a significantly higher proportion of patients achieved HbA_{1c} \leq 6.5% (53.8% vs 41.2%) with no increase in the hypoglycemic event rate in the combination group compared with the metformin monotherapy group.

Applications

Evidence suggests that patients with type 2 diabetes mellitus (T2DM) increasingly require multiple pharmacological combinations to reach treatment goals. In clinical practice, metformin is up-titrated up to maximum doses before adding a second antidiabetic. An early combination, using two oral anti-diabetic drugs with complementary mechanisms of action is an alternative approach that may provide better or more sustained glycemic control with better tolerability. Our approach to earlier aggressive treatment with vildagliptin add on to metformin in patients with T2DM could be of interest in patients like elderly populations (> 60 years of age), as well as patients susceptible to GI events.

Terminology

Glucagon-like peptide-1 (GLP-1) has been shown to increase insulin secretion and suppress glucagon release in a glucose-dependent manner. However, active circulating GLP-1 has a half-life of approximately 1 to 2 min and is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to an inactive state. Inhibition of DPP-4 with vildagliptin, a selective DPP-4 inhibitor, results in enhanced active GLP-1 levels *in vivo*.

Peer review

The manuscript was well prepared. The study was well designed and performed. The data were solid to support the hypothesis and conclusion.

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Mohammad Abdollahi, Professor, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1417614411, Iran

Lu Cai, PhD, Associate Professor, Department of Pediatrics,

University of Louisville School of Medicine, 570 South Preston Street, Suite 304F, Louisville, KY 40202, United States

Ugur Cavlak, Professor, Pamukkale Universitesi Fizik Tedavi ve Rehab, Yuksekokulu Kinikli Kampusu Yeni Rektörlük Binasi B Kati 20070 Denizli, Turkey

Cevdet Kaya, MD, Associate Professor, Haydarpasa Numune Training and Research Hospital, Department of Urology, Kadikoy, Istanbul, Turkey

Suresh Mathews, PhD, Assistant Professor, Department of Nutrition and Food Sciences, 260 Lem Morrison Dr., 101 PSB, Auburn University, Auburn, AL 36849, United States



Meetings

Events Calendar 2010

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 28-30
Hong Kong, China
The 1st International Congress on
Abdominal Obesity

March 09-12
Brussels, Belgium
30th International Symposium on
Intensive Care and Emergency
Medicine

March 23-26
Cairo, Egypt
14th Pan Arab Conference on
Diabetes PACD14

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual
Meeting

June 09-12
Singapore, Singapore
13th International Conference on
Emergency Medicine

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific
Sessions

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference
on Antimicrobial Agents and
Chemotherapy Annual Meeting



Instructions to authors

GENERAL INFORMATION

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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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- 12 **Breedlove GK**, Schorheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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- 15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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