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Inhibition of dipeptidyl peptidase IV activity by oral metformin in Type 2 diabetes

J. R. Lindsay*, N. A. Duffy†, A. M. McKillop†, J. Ardill‡, F. P. M. O'Harte†, P. R. Flatt† and P. M. Bell*

*Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, †School of Biomedical Sciences, University of Ulster, Coleraine, and ‡Wellcome Laboratory, The Queen's University of Belfast, Belfast, Northern Ireland, UK

Accepted 1 June 2004

Abstract

Aims Glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are important insulinotropic hormones that enhance the insulin secretory response to feeding. Their potential for treating Type 2 diabetes is limited by short biological half-life owing to degradation by dipeptidyl peptidase IV (DPP IV). We investigated the acute effects of metformin on DPP IV activity in Type 2 diabetes to elucidate inhibition of DPP IV as a possible mechanism of action.

Methods Eight fasting subjects with Type 2 diabetes (5M/3F, age 53.1 ± 4.2 years, BMI 36.8 ± 1.8 kg/m^2, glucose 8.9 ± 1.2 mmol/l, HbA_{1c} 7.8 ± 0.6%) received placebo or metformin 1 g orally 1 week apart in a random, crossover design.

Results Following metformin, DPP IV activity was suppressed compared with placebo ($AUC_{0-6\,h}$ 3230 ± 373 vs. 5764 ± 504 nmol ml/l, respectively, $P = 0.001$). Circulating glucose, insulin and total GLP-1 were unchanged. Metformin also concentration-dependently inhibited endogenous DPP IV activity *in vitro* in plasma from Type 2 diabetic subjects.

Conclusion Oral metformin effectively inhibits DPP IV activity in Type 2 diabetic patients, suggesting that the drug may have potential for future combination therapy with incretin hormones.

Diabet. Med. 22, 654–657 (2005)

Keywords dipeptidyl peptidase IV, metformin, GLP-1, Type 2 diabetes

Introduction

Incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are currently under investigation as possible agents for treatment of Type 2 diabetes [1,2]. Potential therapeutic effects include augmentation of insulin secretion as well as inhibition of glucagon secretion and gastric emptying [1]. However, the incretins are metabolized rapidly by dipeptidyl peptidase IV (DPP IV), an enzyme widely distributed in plasma and most tissues [3]. Dipeptidyl peptidase IV is responsible for N-terminal degradation of GLP-1 and GIP, by cleavage of N-terminal di-peptide residues yielding biologically inactive peptide fragments [3]. Attempts to produce suitable therapies for use in Type 2 diabetes have focused on development of degradation-resistant incretin analogues or use of novel DPP IV inhibitors [1,2,4].

Currently there is much interest in the possible effects of metformin on DPP IV activity, circulating GLP-1 and the enteroinsular axis [5–7]. Mannucci *et al.* reported increased active GLP-1 concentrations following an oral glucose load in Type 2 diabetic patients treated with metformin [5]. Although enhanced GLP-1 secretion cannot be ruled out, increased GLP-1 concentrations were linked to inhibition of GLP-1 degradation by metformin, as demonstrated *in vitro* using pooled human serum or buffer containing purified DPP IV [5]. Another study described additive glucose-lowering effects of GLP-1 and metformin in Type 2 diabetic patients [6], but no significant differences in circulating active or total GLP-1 were observed [6]. In contrast, observations in DPP IV-deficient rats indicated raised levels of active GLP-1 following administration of metformin in the fasted state [7]. These authors also questioned significant inhibition of DPP IV by metformin, at least *in vitro* [7].

Correspondence to: Prof. P. M. Bell, 1st Floor, East Wing, Royal Victoria Hospital, Belfast, BT12 6BA, Northern Ireland, UK.
E-mail: patrick.bell@royalhospitals.n-i.nhs.uk

This study was designed to investigate the effects of an acute dose of metformin on DPP IV activity and total GLP-1 concentrations compared with placebo in Type 2 diabetes. In addition, we have evaluated the *in vitro* effects of metformin on DPP IV activity using plasma from patients with Type 2 diabetes.

Methods

Subjects and protocol

Eight patients with Type 2 diabetes (five males and three females, age 53.1 ± 4.2 years, duration of diabetes 2.5 ± 1.3 years, BMI 36.8 ± 1.8 kg/m^2 and HbA_{1c}, 7.8 ± 0.6%) were recruited from clinics at the Royal Victoria Hospital, Belfast. All were treated as outpatients with dietary therapy. The Queen's University of Belfast Ethics Committee approved the study and all subjects gave written informed consent. Patients fasted from 22:00 h on the evening preceding the investigation. An intravenous cannula was sited at the antecubital fossa for blood sampling at the times shown in Fig. 1. Each patient was studied on two occasions in a randomized, crossover design, receiving either placebo or 1 g of metformin (Merck Pharmaceuticals, West Drayton, UK) on separate study mornings 1 week apart. A final sample was taken after non-fasting 24 h from the start of the protocol.

Biochemical assays

Dipeptidyl peptidase IV activity was determined [8] in triplicate using a fluorometric method for measurement of free AMC (7-amino-4-methyl-coumarin) liberated from the DPP IV substrate, Gly-Pro-AMC. An aliquot of plasma or purified DPP IV enzyme (10 µl) was incubated for 60 min at 37 °C with 25 µl of 50 mmol HEPES buffer (pH 7.4) containing the Gly-Pro-AMC substate (final substrate concentration 1 mmol/l). Where appropriate, metformin was incorporated into the 25 µl of HEPES buffer at the concentrations stated in Fig. 2. The reaction was stopped by addition of 70 µl of 3 mol/l acetic acid. AMC was measured by comparison with a standard curve (range 3.9–500 nmol/l) by fluorimetric assay using excitation and emission wavelengths of 370 nm and 440 nm, respectively, using a Flexstation (Molecular Devices, Crawley, West Sussex, UK). Results of DPP IV activity are displayed as nmol/ml/min. The intra and interassay coefficients of variation of the assay were 2.1% and 6.9%, respectively. Glucose concentrations were determined using a glucose-oxidase method. HbA_{1c} was measured in whole blood by ion-exchange HPLC. Serum insulin was determined using a microparticulate enzyme immunoassay (Abbott Laboratories Ltd, Berkshire, UK). Glucagon-like peptide-1 was measured by a competitive C-terminally directed radioimmunoassay, using rabbit antibody R600-8 (Bachem; Department of Medicine, Queen's University, Belfast, UK) with charcoal separation of bound and free moieties.

Statistical analysis

Significant differences between groups of data were assessed using repeated measures ANOVA with Greenhouse Geisser-corrected *F*-tests. Area under the curve (AUC) was calculated using the trapezoidal rule between $t = 0$ to $t = 6$ h and compared using a Student's paired *t*-test. Observations at each time point were similarly compared in the event of a statistically significant interaction between treatment and time arising in the ANOVA. Statistical significance was assumed if $P < 0.05$.

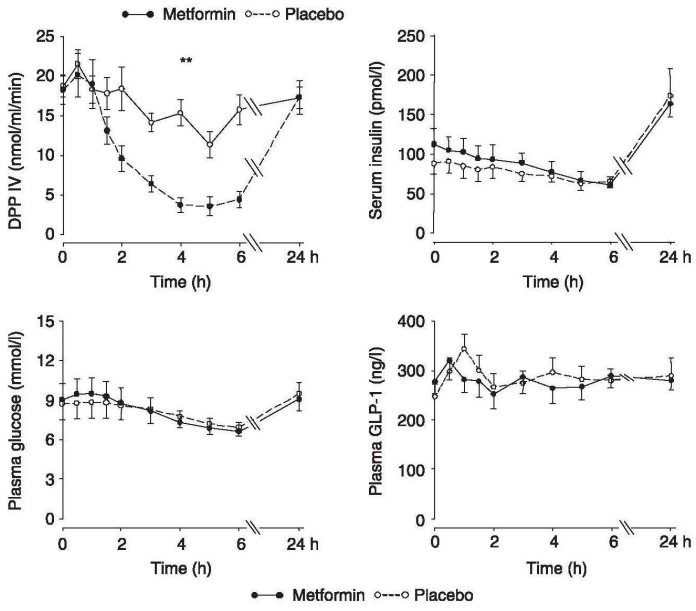

Figure 1 Effects of metformin 1 g orally compared with placebo on circulating dipeptidyl peptidase IV (DPP IV) activity, glucose, insulin and total glucagon-like peptide-1 (GLP-1) responses in Type 2 diabetic patients. Values are mean ± SEM for eight subjects (**$P < 0.01$ metformin compared with placebo using $AUC_{t=0-6\,h}$).

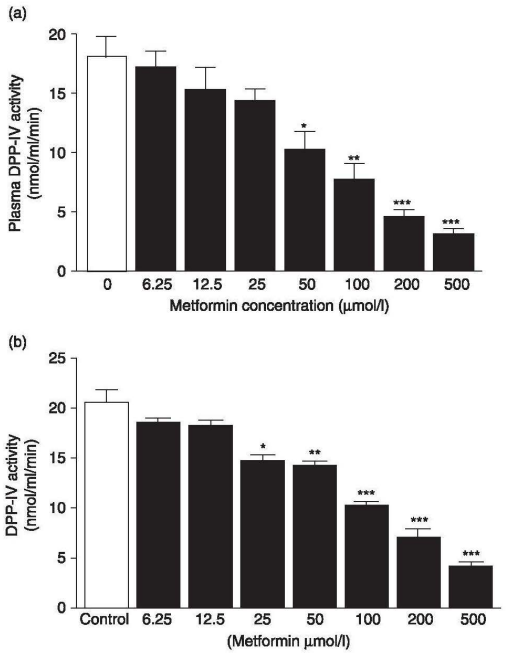

Figure 2 *In vitro* effects of metformin on dipeptidyl peptidase IV (DPP IV) activity in (a) plasma from Type 2 diabetic subjects and (b) purified DPP IV enzyme. Values are mean ± SEM for three subjects. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with activity in the absence of added metformin.

Results

Circulating DPP IV, glucose and hormone profiles following metformin and placebo

Dipeptidyl peptidase IV activity at baseline was similar for the metformin and placebo days (Fig. 1). Following metformin there was rapid suppression compared with the placebo across 6 h as measured by $AUC_{t=0-6\,h}$ (3230 ± 373 and 5764 ± 504 nmol/ml, respectively, $P = 0.001$). Dipeptidyl peptidase IV activity following metformin fell from baseline to a nadir of 4 ± 1 nmol/ml/min at 5 h, which was lower than the nadir of 11 ± 2 following the placebo ($P < 0.0001$). Dipeptidyl peptidase IV activity 24 h following metformin had returned to 17 ± 6 nmol/ml/min, which was comparable to basal levels and those 24 h after the placebo (17 ± 4 nmol/ml/min). Fasting glucose, insulin and GLP-1 levels were comparable for the placebo and metformin days. ANOVA showed no differences in these parameters in the 6 h following metformin compared with placebo (Fig. 1).

Metformin effects on endogenous DPP IV activity in Type 2 diabetic plasma *in vitro* and on purified DPP IV activity

Metformin concentration-dependently inhibited endogenous DPP IV activity (IC_{50} 56 μmol/l) in plasma from subjects with Type 2 diabetes (Fig. 2A). The effect was significant by 50 μmol/l of metformin and increased to an observed maximal 84% inhibition at the highest concentration of metformin tested (500 μmol/l). We can report that metformin also causes a dose-dependent decrease in DPP IV activity in plasma from healthy individuals (which starts with higher basal DPP IV activity compared with diabetic subjects) with an approximate IC_{50} value of 80 μmol/l (data not shown). Similarly, metformin caused a dose-dependent inhibition of purified DPP IV enzyme activity (IC_{50} 98 μmol/l, Fig. 2B), which was significantly decreased (by 28%, $P < 0.05$) at a concentration of 25 μmol/l and with a maximal inhibition (80%) occurring at 500 μmol/l.

Discussion

Therapeutic strategies proposed to enhance the incretin effect by administration of GLP-1 or GIP are under consideration for Type 2 diabetes [1,2]. However, this approach is frustrated by the short half-lives of these peptides owing to rapid degradation by DPP IV [3,4]. One way of circumventing this difficulty is to develop stable, long-acting DPP IV-resistant analogues. N-terminal analogues of both GIP and GLP-1 have been shown to exhibit resistance to DPP IV *in vitro* and prolong insulinotropic activity *in vivo* in Type 2 diabetes [1,2]. N-acetyl and N-pyroglutamyl analogues of GIP plus the GLP-1 analogue NN2211 appear to be particularly effective *in vivo*, raising the possibility of their use in therapy of Type 2 diabetes mellitus [1,2].

An alternative strategy to enhance the action of GLP-1 and GIP is the use of DPP IV inhibitors, which decrease peptide degradation and increase the physiological incretin effect [4]. Inhibitors tested both *in vitro* and *in vivo* include isoleucine thiazolidide (P32/98), valine pyrrolidine, FE99901 and NVP-DPP728, which result in improved insulin secretion and glucose tolerance in animals and humans. Although, many other bioactive peptides are substrates for DPP IV [3], this approach might be useful in promoting endogenous incretin hormone effects or for prolongation of biologically active GLP-1 or GIP given by infusion [4].

In contrast to these novel approaches, metformin has been available for treating diabetes since the 1950s [9]. Metformin improves insulin sensitivity and reduces hepatic glucose output and gluconeogenesis. The current study demonstrated significant inhibitory effects on circulating DPP IV activity in Type 2 diabetic patients between 1.5 and 6 h after administration, consistent with the T_{max} for metformin. Following a 1-g dose the metformin concentration and effective dose in plasma of diabetic subjects would be expected to reach approximately 20 μmol/l [10]. The *in vitro* study that examined the dose-dependent effect of metformin on DPP IV activity (Fig. 2B) demonstrated that a comparable concentration of 25 μmol/l metformin (18 μmol/l in final reaction mixture) is effective at inhibiting DPP IV activity. Return of DPP IV activity to basal levels was observed within 24 h, suggesting an escape from the

inhibitory effects of metformin from 6 h after drug administration. To achieve more sustained inhibition of DPP IV activity, metformin dosing regimens of two or three times daily may be required.

It is not certain if the acute effects of metformin on DPP IV activity demonstrated in the present study will be reproducible in chronic dosing regimens in Type 2 diabetes [5]. Nevertheless our data support a new mechanism of metformin action as suggested previously [5]. The inability of others to reproduce such effects [7,11] is difficult to explain but may relate to the use of alternative assay methodologies. Interestingly, there is evidence also that chronic inhibition of DPP IV activity may delay the onset of diabetes in animal models of impaired glucose tolerance [12]. Together with the present observations, therapeutic inhibition of DPP IV activity may partly explain the mechanism of metformin action in diabetes prevention in man [13].

As expected, metformin given in the fasting state did not acutely lower circulating glucose or increase insulin concentrations [9]. However, our study design did allow analysis of responses of DPP IV activity to metformin without the influence of other variables. An additional limitation to the current study was the lack of an available N-terminally directed assay to detect biologically active GLP-1. Glucagon-like peptide-1 levels determined by a C-terminally directed assay measuring active and cleaved inactive fragments remained unchanged following metformin. This would not support a direct stimulatory effect of metformin on GLP-1 secretion as suggested by observations in DPP IV-deficient rats [7]. However, further studies testing metformin under conditions of entero-endocrine nutrient stimulation are required to clarify this proposal [11].

In conclusion, this study has demonstrated that oral metformin inhibits DPP IV activity acutely in patients with Type 2 diabetes. Although there was no antihyperglycaemic or insulinotropic effects in the fasting state, metformin may contribute to glycaemic control by reducing the degradation of enteroinsular hormones secreted following feeding. Knowing that metformin inhibits DPP IV activity supports the possible use of metformin in combination with incretin hormones for therapy in Type 2 diabetes.

Acknowledgements

We thank Dr Chris Patterson for statistical advice. These studies were supported by the R & D Office of the Department of Health and Personal Social Services (NI) and University of Ulster research strategic funding. JRL received a R & D Office Research Fellowship and NAD received a R & D Office Research Studentship.

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