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Addition of metformin to exogenous glucagon-like peptide–1 results in increased serum glucagon-like peptide–1 concentrations and greater glucose lowering in type 2 diabetes mellitus

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Abstract

Glucagon-like peptide–1 (GLP-1) is an incretin hormone that lowers blood glucose after meals in type 2 diabetes mellitus. The therapeutic potential of GLP-1 in diabetes is limited by rapid inactivation by the enzyme dipeptidylpeptidase-4 (DPP-4). Metformin has been reported to inhibit DPP-4. Here we investigated the acute effects of metformin and GLP-1 alone or in combination on plasma DPP-4 activity, active GLP-1 concentrations, and glucose lowering in type 2 diabetes mellitus. Ten subjects with type 2 diabetes mellitus (8 male and 2 female; age, 68.7 ± 2.6 years [mean ± SEM]; body mass index, 29.6 ± 1.7 kg/m^2; hemoglobin A_{1c}, 7.0% ± 0.1%) received 1 of 3 combinations after an overnight fast in a randomized crossover design: metformin 1 g orally plus subcutaneous injection saline (Metformin), GLP-1 (1.5 nmol/kg body weight subcutaneously) plus placebo tablet (GLP-1), or metformin 1 g plus GLP-1(Metformin + GLP-1). At 15 minutes, glucose was raised to 15 mmol/L by rapid intravenous infusion of glucose; and responses were assessed over the next 3 hours. This stimulus does not activate the enteroinsular axis and secretion of endogenous GLP-1, enabling the effect of exogenously administered GLP-1 to be examined. Mean area under curve (AUC) $_{0\text{-}180\ \text{minutes}}$ plasma glucose responses were lowest after Metformin + GLP-1 (mean ± SEM, 1629 ± 90 mmol/[L min]) compared with GLP-1 (1885 ± 86 mmol/[L min], $P < .002$) and Metformin (2045 ± 115 mmol/[L min], $P < .001$). Mean AUC serum insulin responses were similar after either Metformin + GLP-1 (5426 ± 498 mU/[L min]) or GLP-1 (5655 ± 854 mU/[L min]) treatment, and both were higher than Metformin (3521 ± 410 mU/[L min]; $P < .001$ and $P < .05$, respectively). Mean AUC for plasma DPP-4 activity was lower after Metformin + GLP-1 (1505 ± 2 μmol/[mL min], $P < .001$) and Metformin (1508 ± 2 μmol/[mL min], $P < .002$) compared with GLP-1 (1587 ± 3 μmol/[mL min]). Mean AUC measures for plasma active GLP-1 concentrations were higher after Metformin + GLP-1 (820 × 10^4 ± 51 × 10^4 pmol/[L min]) compared with GLP-1 (484 × 10^4 ± 31 × 10^4 pmol/[L min], $P < .001$) and Metformin (419 × 10^4 ± 34 × 10^4 pmol/[L min], $P < .001$), respectively. In patients with type 2 diabetes mellitus, metformin inhibits DPP-4 activity and thus increases active GLP-1 concentrations after subcutaneous injection. In combination with GLP-1, metformin significantly lowers plasma glucose concentrations in type 2 diabetes mellitus subjects compared with GLP-1 alone, whereas insulin responses were similar. Metformin enhances serum concentrations of injected active GLP-1(7-36)amide, and the combination results in added glucose-lowering potency.

1. Introduction

Despite significant advances in the management of diabetes, optimal glycemic control is often not achieved or maintained. Hypoglycemia and weight gain associated with many antidiabetic medications may interfere with the implementation and long-term application of "intensive" therapies [1]. All current treatments for type 2 diabetes mellitus have important limitations, and the search for new compounds to provide alternative and/or additional glucose-lowering capacity continues.

Much attention has focused on manipulating the therapeutic potential of the enteroinsular axis that is mediated through the incretin hormones glucagon-like peptide–1

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(GLP-1) and glucose-dependent insulinotropic peptide [2]. As well as stimulating insulin secretion, GLP-1 decreases glucose levels by decreasing glucagon secretion, delaying gastric emptying, and reducing food intake. It may enhance insulin sensitivity [3] and, by stimulating β-cell regeneration and inhibition of apoptosis [4], holds out the prospect of altering the natural history of type 2 diabetes mellitus. It acts in a glucose-dependent manner, thus significantly reducing the risk of hypoglycemia [5]. Therefore, as a therapeutic agent, GLP-1 has several potential advantages over current treatments. It has been shown to lower glucose effectively by regular subcutaneous injection [6] and by intravenous [7] or subcutaneous infusion [8]. However, the circulating half-life of GLP-1 is about 2 to 3 minutes [9] because it is broken down by the enzyme dipeptidylpeptidase-4 (DPP-4) [10], limiting its use in routine clinical practice. Several strategies have been explored to help prolong GLP-1 or GLP-1–like action. Influencing the enzyme DPP-4 is a key target of several recently developed pharmacologic treatments. These include the DPP-4 antagonists and DPP-4–resistant analogues of GLP-1 [3].

In previous studies, we have shown that metformin has a small but consistent effect on inhibiting DPP-4 activity. In the present study, we aimed to explore the ability of DPP-4 inhibition by metformin to prolong the activity and enhance the biological effectiveness of exogenously administered GLP-1.

2. Materials and methods

2.1. Subjects

This study was undertaken in 10 subjects with type 2 diabetes mellitus (8 male and 2 female; age, 68.7 ± 2.6 years [mean ± SEM]; hemoglobin A_{1c}, 7.0% ± 0.1%). They were all recruited from the diabetic outpatient clinics at the Royal Victoria Hospital, Belfast; and the World Health Organization criteria for type 2 diabetes mellitus at diagnosis were used [11]. Exclusion criteria included being treated with insulin, requiring 2 or more oral hypoglycemic agents, or hemoglobin A_{1c} greater than 7.5%. Two subjects were diet controlled, and 8 required 1 oral hypoglycemic agent for control of their diabetes. Also excluded were those with significant renal impairment (serum creatinine >150 μmol/L), or liver or cardiac disease. The Northern Ireland Health and Personal Social Services Research Ethics Committee approved the study, and all subjects gave written informed consent.

2.2. Study protocol

Oral hypoglycemic agents were discontinued 3 weeks before participation. Each subject was studied on 3 occasions 1 week apart. Subjects fasted from 10:00 PM on the evening preceding the investigation and attended the Endocrinology and Diabetes Research Centre, Royal Victoria Hospital, at 8:00 AM. With patients in the recumbent position, an intravenous cannula was inserted in the antecubital fossa for blood sampling at −10, 0, 15, 30, 60, 90, 135, and 180 minutes. A second intravenous cannula was inserted in the other antecubital fossa for the administration of intravenous glucose. At time 0 minute, subjects received in a randomized crossover design 1 of 3 combinations:

1. Metformin 1 g orally plus placebo by subcutaneous injection.
2. GLP-1 1.5 nmol/kg body weight subcutaneously (SC) (GLP-1[7-36]amide supplied as a 3-mL, 1.0-mg/mL liquid sterile formulation; Restoragen, Lincoln, NE) plus placebo tablet.
3. Metformin 1 g orally plus GLP-1 (1.5 nmol/kg body weight SC).

At 15 minutes, glucose was administered by rapid intravenous injection to raise plasma glucose to 15 mmol/L (this stimulus was chosen so as not to activate the enteroinsular axis and secretion of endogenous GLP-1, enabling the hypoglycemic effect of metformin and exogenously administered GLP-1 to be examined). The amount of glucose infused to raise plasma glucose to 15 mmol/L was calculated using fasting plasma glucose and body weight [12], as follows:

$$(15\,\text{mmol/L} - \text{fasting plasma glucose mmol/L}) \times (35\,\text{mg glucose}) \times (\text{body weight in kg})$$

Blood was withdrawn and analyzed for glucose, insulin, C-peptide, and GLP-1 concentrations and DPP-4 activity. At the end of the study period, in those subjects previously taking oral hypoglycemic agents, medication was restarted; and all were served lunch.

2.3. Biochemical assays

Glucose concentrations were determined using a glucose oxidase method [13]. Serum insulin and C-peptide were determined at the Regional Endocrinology and Biochemistry laboratories at the Royal Victoria Hospital, Belfast, using standard commercial kits.

The DPP-4 activity was determined in triplicate using an assay developed at the University of Ulster, Coleraine, as previously described [14-16]. Briefly, it consisted of a fluorometric method for the measurement of free 7-amino-4-metforminhyl-coumarin (AMC) liberated from the substrate Gly-Pro-AMC by an enzymatic reaction with DPP-4. *One unit of DPP-4 activity* was defined as the enzyme activity that produced 1 nmol of AMC per 10 μL of plasma in 1 minute. Active GLP-1 was measured using a commercial enzyme-linked immunoassay kit (Linco Research, St Charles, MO). The intra- and interassay coefficients of variation were 6% to 8% and 7% to 13%, respectively.

2.4. Statistics

Area under curve (AUC) was used to give an integrated measure of responses. Areas were calculated using the trapezoidal rule with baseline subtraction and compared using Student *t* test for paired data. Observations at each time point were similarly compared in the event of a statistically significant interaction between treatment and time arising in the analysis of variance. Statistical significance was assumed if $P < .05$. From our earlier clinical study with metformin, we calculated that we required 8 patients to have 90% power at the 5% level of significance of being able to detect a 25% reduction in DPP-4 activity.

3. Results

All 10 patients completed the study. One subject had a mild symptomatic hypoglycemic episode after the combination of 1 g metformin and subcutaneous injection of GLP-1 (Metformin + GLP-1).

Plasma glucose responses were lowest after the combination of Metformin + GLP-1 (1629 ± 90 mmol/[L min]) (mean $AUC_{0\text{-}180\ minutes}$ ± SEM) compared with GLP-1 (1885 ± 86 mmol/[L min], $P < .002$) or Metformin (2045 ± 115 mmol/[L min], $P < .001$). At 60 minutes, mean plasma glucose levels were also lowest after Metformin + GLP-1 (8.8 ± 0.7 mmol/L) compared with GLP-1 (10.9 ± 0.5 mmol/L, $P < .001$) or Metformin (12.4 ± 0.7 mmol/L, $P < .001$) (Fig. 1).

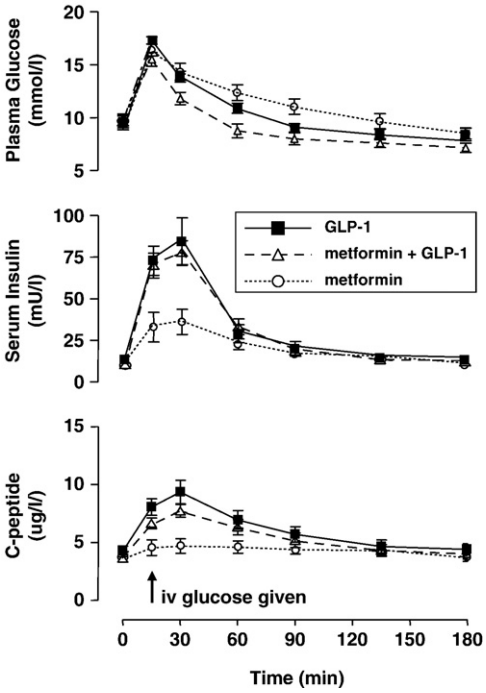

Fig. 1. Effect of metformin and GLP-1 (SC) on plasma glucose, serum insulin, and C-peptide in fasting patients with intravenous glucose given at 15 minutes.

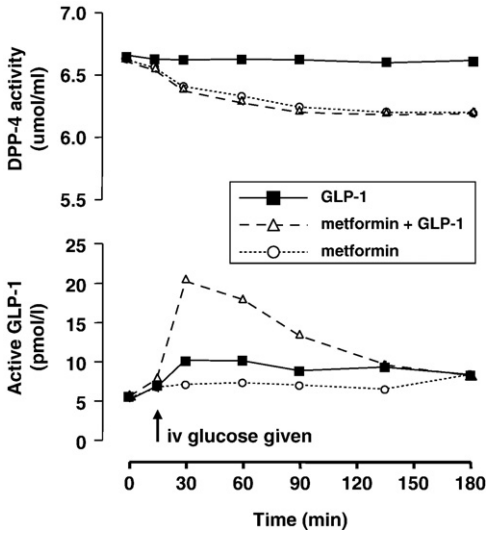

Fig. 2. Effect of metformin and GLP-1 (SC) on serum DPP-4 activity and GLP-1 concentrations in fasting patients with intravenous glucose given at 15 minutes.

Mean AUC serum insulin responses (Fig. 1) were similar after either Metformin + GLP-1 (5426 ± 498 mU/[L min]) or GLP-1 (5655 ± 854 mU/[L min]) treatment, but both were higher than Metformin (3521 ± 410 mU/[L min]; $P < .001$ and $P < .001$, respectively). Serum C-peptide (Fig. 1) responses were comparable to the insulin responses with mean $AUC_{0\text{-}180\ minutes}$ after both Metformin + GLP-1 (967 ± 91 μg/[L min]) and GLP-1 (1080 ± 118 μg/[L min]) treatment being higher than Metformin (781 ± 77; $P < .001$ and $P < .001$, respectively).

Plasma DPP-4 activity was slightly but consistently lower after Metformin + GLP-1 ($AUC_{0\text{-}180\ minutes}$ 1505 ± 2 μmol/[mL min]) or Metformin (1508 ± 2 μmol/[mL min]) compared with GLP-1 (1587 ± 3 μmol/[mL min]; $P < .001$ and $P < .001$, respectively) (Fig. 2).

Plasma active GLP-1 concentrations were higher after Metformin + GLP-1 ($AUC_{0\text{-}180\ minutes}$ 2281 ± 29 pmol/[L min]) compared with GLP-1 (1596 ± 28 pmol/[L min], $P < .001$) and Metformin (1247 ± 26 pmol/[L min], $P < .001$) (Fig. 2).

4. Discussion

This study allowed the assessment of the short-term effects of metformin on DPP-4 activity when given either alone or with GLP-1. As subjects were kept fasting, there was no endogenous enteroinsular stimulation, thus allowing the effects of exogenously administered GLP-1 to be assessed.

Plasma glucose levels were significantly reduced after the combination of oral metformin and GLP-1 compared with

either GLP-1 or metformin alone. Despite the difference in blood glucose, serum insulin profiles were similar after combination of metformin and GLP-1 compared with GLP-1 alone but both were significantly higher than after metformin alone. These results are consistent with the well-recognized insulinotropic effects of GLP-1 [17].

The DPP-4 activity after combination of metformin and GLP-1 as well as after metformin alone was significantly lower than with GLP-1 alone. This supports earlier work in animal and human studies that metformin has the ability to effectively inhibit DPP-4 activity. Green and colleagues [16] demonstrated that in vivo metformin lowered plasma DPP-4 activity in obese diabetic (*ob/ob)* mice and improved glucose-lowering and insulin-releasing effects of exogenous GLP-1. In fasting subjects with type 2 diabetes mellitus, Lindsay et al showed that metformin significantly suppressed DPP-4 activity [14]. In the present study, as might be anticipated with DPP-4 activity being reduced by metformin, active GLP-1 concentrations after metformin and GLP-1 combination were significantly higher compared with GLP-1 alone.

It is generally assumed that the major glucose-lowering effect of GLP-1 is by stimulating insulin secretion. However, in our study, although plasma GLP-1 concentrations were significantly higher with combined metformin and GLP-1 compared with GLP-1 alone, insulin responses were almost identical. Despite this, plasma glucose was significantly lower after the combination of metformin and GLP-1 compared with either GLP-1 or metformin alone. This suggests either that GLP-1 lowered glucose by mechanisms other than increased insulin release or, alternatively, that additional insulin-sensitizing effects of metformin played a part. Others have demonstrated in animals and man that supraphysiologic levels of GLP-1 could increase hepatic glucose uptake and glycogen synthesis independent of an action on insulin or glucagon secretion [18,19]. Glucagon-like peptide–1 also acts on pancreatic α-cells to inhibit glucagon secretion. In individuals with insulin requiring diabetes and no functional secreting β-cells, GLP-1 infusion resulted in lower glucose levels, despite undetectable C-peptide levels, while strongly inhibiting glucagon release [20,21]. When GLP-1 was given by intravenous infusion for 4 to 6 hours to patients with type 2 diabetes mellitus, it lowered glucose by both insulin stimulation and inhibition of glucagon secretion [22]. In human muscle, GLP-1, like insulin, stimulated glycogen synthesis, glycogen synthase a activity, and glucose oxidation and utilization and inhibited glycogen phosphorylase a activity, all of this at physiologic concentrations of the peptide [23]. This work confirms the potent stimulatory effect of GLP-1 on the glucose metabolism of human skeletal muscle independent of insulin. Taken together, these data help explain the finding in our present study in which the combination of metformin and GLP-1 compared with GLP-1 alone both inhibited DPP-4 activity and subsequently raised GLP-1 levels, resulting in a lower plasma glucose but no significant difference in serum insulin concentrations.

A further possibility is that inhibition of DPP-4 augmented the release of other deficient incretins such as glucose-dependent insulinotropic peptide or related peptide hormones such as pituitary adenylate cyclase-activating peptide or gastrin-releasing peptide [24]. Dipeptidylpeptidase-4 inhibition may, in fact, have pleiotropic effects because this enzyme has been shown to inhibit the degradation of over 20 peptides in the human body. Neuropeptides stored in islet nerve terminals are also affected by DPP-4 inhibition, and this could potentially regulate islet function [25,26].

Selective DPP-4 inhibitors used in the management of type 2 diabetes mellitus inhibit DPP-4 activity by 80% over 24 hours [27,28]. In our study metformin inhibited DPP-4 activity by only 7%. Although the selective DPP-4 inhibitors clearly inhibit DPP-4 activity much more than metformin, the extent to which they lower plasma glucose is less [29,30], emphasizing that metformin mainly acts through other glucose-lowering mechanisms. Despite this, the current findings are potentially relevant to the management of type 2 diabetes mellitus particularly with regard to the increasing use of combination therapy. Metformin may have additional beneficial effects over and above its traditional insulin-sensitizing role when used along with incretin hormones.

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