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Combined antidiabetic benefits of exenatide and dapagliflozin in diabetic mice†

The combined glucose-lowering effect of exenatide and dapagliflozin has not yet been studied. We investigated this combination (single-dose or 4-week dosing) in diabetic *ob/ob* mice. Vehicle-corrected basal glucose showed greater reduction 1 h following exenatide + dapagliflozin than with exenatide or dapagliflozin alone, and stayed significantly lower for all groups versus vehicle over 3 h. During an oral glucose tolerance test, glucose excursion (30 min post-dose) was significantly lower for exenatide + dapagliflozin versus exenatide or dapagliflozin, or vehicle. Exenatide + dapagliflozin and exenatide, but not dapagliflozin alone, reduced glucose excretion over 24 h versus vehicle. After dosing for 4 weeks, exenatide, dapagliflozin and exenatide + dapagliflozin similarly decreased haemoglobin A1c (HbA1c). Body weight was reduced only with exenatide or exenatide + dapagliflozin. The glomerular filtration rate was similar with exenatide, dapagliflozin and vehicle, and increased with exenatide + dapagliflozin. Optimized combinatorial dosing of these antidiabetic agents may provide additive glucose lowering in type 2 diabetes mellitus.
Keywords: combination, dapagliflozin, diabetes, exenatide, glucagon-like peptide-1, mouse

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RESEARCH LETTER

Introduction

Exenatide is an incretin-based treatment for type 2 diabetes mellitus (T2DM). Exenatide maintains glucose homeostasis through glucagon-like peptide-1 (GLP-1) receptor-mediated actions, including glucose-dependent insulinotropism, reduction of food intake and body weight, suppression of glucagon secretion and slowing of gastric emptying [1]. The majority of T2DM patients receiving exenatide [2] achieve recommended HbA1c levels <7% [3], however, relatively few reach values observed in non-diabetic adults (≤5.6%) [4]. Dapagliflozin belongs to a novel class of antidiabetic therapies that regulate glucose levels through inhibition of renal glucose reabsorption, and increase urinary glucose excretion via selective inhibition of the renal sodium glucose cotransporter-2 (SGLT-2) [5,6]. This study tested whether combining dapagliflozin and exenatide improves glucose homeostasis via additive or synergistic effects on glycaemic control, using diabetic, obese *ob/ob* mice.

Methods

Animals

All procedures were conducted in accordance with Animal Welfare Act guidelines and approved by the Amylin IACUC. Male 8-week-old leptin-deficient B6.V-Lep^{ob}/J (*ob/ob*) mice (Jackson Laboratories, Bar Harbor, ME, USA; body weight 43 ± 1 g) were used. Animals were housed individually with a 12-h light/dark cycle and *ad libitum* access to food/water. Mice subjected to a basal glucose-lowering test or oral glucose tolerance test (OGTT) were fasted for 4 or 15 h, respectively. Urinary glucose excretion was assessed using individual metabolic cages.

Compounds

Exenatide was reconstituted in 10% dimethyl sulfoxide (DMSO) or 30 mM sodium acetate for acute and sub-chronic studies, respectively. Dapagliflozin (AKAAL Organics, Long Beach, CA, USA) was reconstituted in water. Doses for each compound were sub-maximal, allowing for detection of potential additive or synergistic pharmacological effects.

Acute Studies

Single doses of exenatide, dapagliflozin, exenatide + dapagliflozin or vehicle were evaluated for effects on basal glucose lowering, OGTT, or 24-h urinary glucose excretion. Compounds were administered at $t = 0$ min for basal glucose lowering, and 15 min before oral gavage with 2 g/kg dextrose for OGTT. Urinary glucose excretion was measured to assess dapagliflozin-induced changes alone, or combined with exenatide.

Sub-Chronic Studies

Four-week effects on glucose metabolism with exenatide (0.03 mg/kg/day), dapagliflozin (1 mg/kg/day), exenatide + dapagliflozin or combined vehicles were assessed in *ob/ob* mice. Exenatide was continuously delivered via subcutaneous osmotic minipump. Dapagliflozin was administered once daily via oral gavage.

Correspondence to: David G. Parkes, PhD, Amylin Pharmaceuticals, LLC, 9625 Towne Centre Drive, San Diego, CA, USA.
E-mail: david.parkes@bms.com

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HbA1c and plasma dapagliflozin levels were measured at weeks 2 and 4. Immediately after final blood sampling at week 4, mice were placed in metabolic cages for 17 h for assessment of glomerular filtration rate [GFR = (urine creatinine × urine volume)/(plasma creatinine × urine time collection)].

Biochemical Analyses

Blood glucose (50 µl, orbital sinus, conscious animals) was measured using an AlphaTRAK® blood glucose meter (Abbott Laboratories, Abbott Park, IL, USA). Urine glucose, creatinine and whole blood HbA1c (140 µl) were analysed using an Olympus AU680 clinical analyzer (Olympus America, Irving, TX, USA). Dapagliflozin concentration was measured using an API 4000 Q-TRAP Mass Spectrometer (AB SCIEX, Framingham, MA, USA).

Statistical Analyses

Results are presented as mean ± SEM, analysed using Prism 5® (GraphPad Software, Inc., La Jolla, CA, USA). Single-point data were analysed using one-way analysis of variance, with Tukey's multiple comparison *post hoc* test. Significant between-group differences were assumed for $p < 0.05$.

Results

Acute Studies

Ob/ob mice were severely diabetic: 4 h fasting blood glucose was 34.6 ± 2.2 mmol/l. At 60 min post-dose, exenatide + dapagliflozin produced significantly greater reductions in glucose (−27.9 ± 2.3 mmol/l, $p < 0.05$) than exenatide (−17.7 ± 0.8 mmol/l) or dapagliflozin (−12.2 ± 4.5 mmol/l; Figure 1A) alone. Blood glucose plateaued in the combination group after 1 h; the exenatide and dapagliflozin groups had similar profiles with maximum decreases at 3 h.

Exenatide + dapagliflozin significantly suppressed glucose excursion during a 2-h OGTT and was more effective than either agent alone (Figure 1B). Blood glucose was significantly lower with exenatide versus dapagliflozin (29.1 ± 1.8 vs.

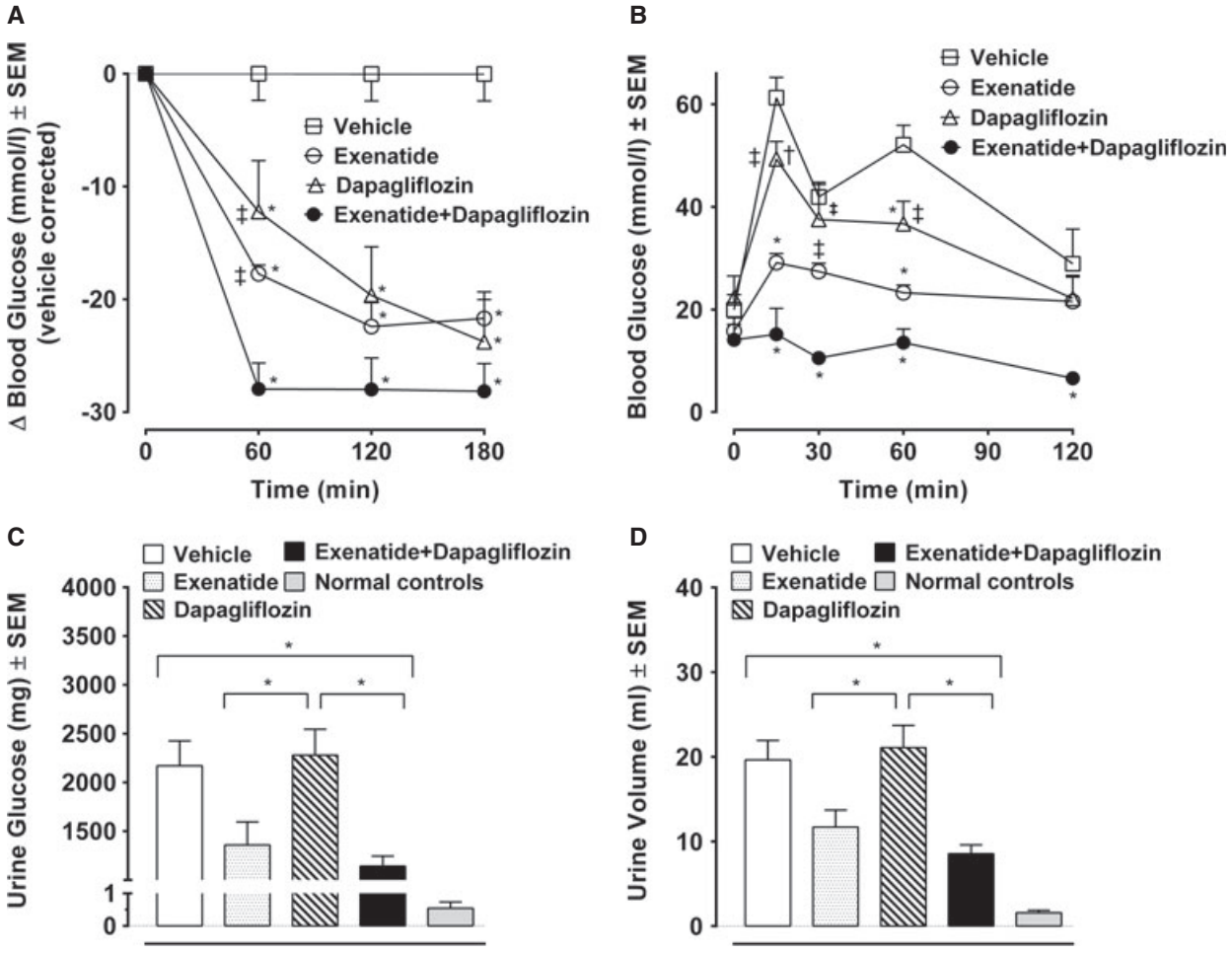

Figure 1. Change in plasma glucose (A) and glucose excursion during an oral glucose tolerance test (B), and total 24-h urine glucose (C) and total urine volume (D) in diabetic *ob/ob* mice after single-dose administration of exenatide (0.03 mg/kg), dapagliflozin (1 mg/kg), exenatide + dapagliflozin combination or combined respective vehicles (vehicle). Data are presented as mean ± s.e.m. $^{*}p < 0.05$ versus vehicle control, $^{\dagger}p < 0.05$ versus exenatide, $^{\ddagger}p < 0.05$ versus exenatide + dapagliflozin.

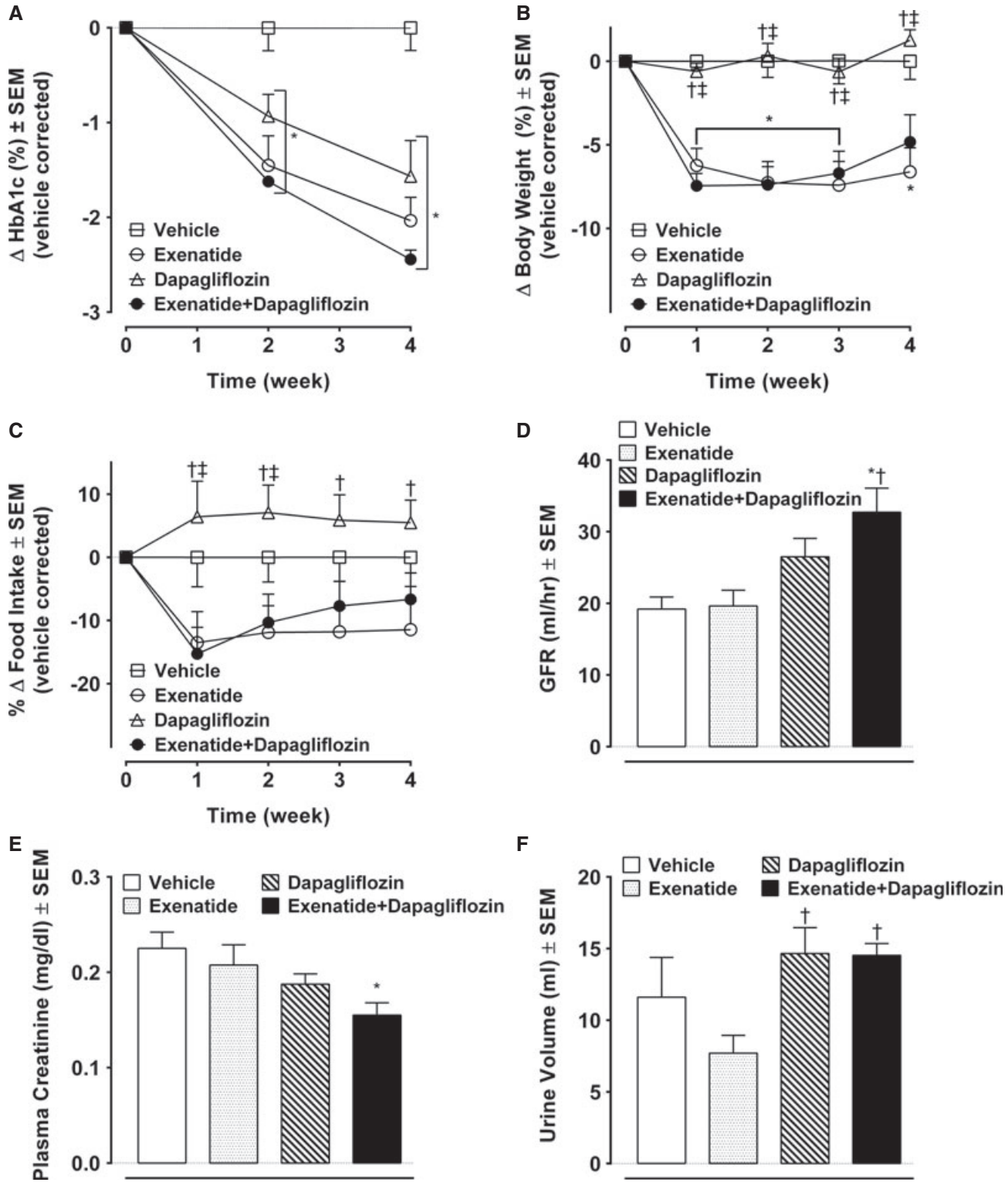

Figure 2. Effects of 4-week administration of exenatide (0.03 mg/kg/day), dapagliflozin (1 mg/kg/day), exenatide + dapagliflozin combination or combined respective vehicles (vehicle) on haemoglobin A1c (HbA1c) change from baseline (A), body weight (B) and food intake (C) change from baseline, glomerular filtration rate (GFR) (D), plasma creatinine (E) and total urine volume (F) in diabetic *ob/ob* mice. Mice were placed in metabolic cages 1 h after the final dapagliflozin dose at week 4, and samples for measurement were then collected over 17 h. Data are presented as mean ± s.e.m. $^{*}p < 0.05$ versus vehicle control, $^{\dagger}p < 0.05$ versus exenatide, $^{\ddagger}p < 0.05$ versus exenatide + dapagliflozin.

49.3 ± 3.4 mmol/l) 15 min post-glucose challenge, but not thereafter.

Dapagliflozin had no significant effect on 24-h total excreted urinary glucose or total urine volume, while exenatide significantly reduced total urine glucose and volume versus vehicle (Figure 1C, D). Exenatide + dapagliflozin also lowered total urine glucose and volume versus vehicle or dapagliflozin alone.

Sub-Chronic Studies

In vehicle-treated mice, HbA1c increased from 7.34 ± 0.01% to 9.09 ± 0.40% during the 4-week study. Administration of exenatide, dapagliflozin or the combination significantly reduced HbA1c versus vehicle at study end (Figure 2A); all treatment groups had similar declines, with the combination showing a trend towards being most effective. Exenatide + dapagliflozin and exenatide alone decreased body weight (Figure 2B). Dapagliflozin alone increased food intake (Figure 2C).

Exenatide + dapagliflozin significantly increased GFR (Figure 2D) and decreased plasma creatinine (Figure 2E), without significant effects on urine volume (Figure 2F) or total urine creatinine. Exenatide or dapagliflozin alone did not modify GFR, urine volume or urine and plasma creatinine levels after 4-week treatment.

Plasma dapagliflozin concentrations were comparable between dapagliflozin (1.24 ± 0.15 and 0.81 ± 0.08 μg/ml) and exenatide + dapagliflozin (1.13 ± 0.04 and 0.65 ± 0.06 μg/ml) at weeks 2 and 4, respectively.

Discussion

This study in diabetic mice reports the novel observation that GLP-1 receptor agonism with exenatide plus SGLT-2 inhibition with dapagliflozin has the potential for additive effects in T2DM. Assessment of basal glucose lowering and oral glucose tolerance showed that exenatide + dapagliflozin controlled blood glucose significantly better than each monotherapy. Longer-term combination therapy significantly reduced HbA1c versus vehicle, with a trend towards greater efficacy than either agent alone.

The apparent additive glucose-lowering effect seen with exenatide + dapagliflozin in the acute studies did not significantly translate to additive HbA1c lowering or weight loss in the sub-chronic study. Several factors may contribute to this discrepancy. First, HbA1c did not reach a plateau in any group by study end; therefore, the sub-chronic study could have been too short to provide definitive results. Second, the chronic dosing regimen for dapagliflozin might not have been optimal. Since *ob*/*ob* mice are hyperphagic, chronic administration of dapagliflozin in food could provide improved exposure/effectiveness. Detailed, multiple dose-interaction studies may be required (e.g. response surface methodology) to elucidate optimal long-term dose combinations that may reveal potential additivity or synergy.

In this study, dapagliflozin acutely lowered blood glucose but did not increase 24-h urinary glucose excretion. Diabetic *ob*/*ob* mice are already polyuric/glucosuric; therefore, further enhancing glucosuria with dapagliflozin may not have been feasible. Dapagliflozin-induced increases in excreted glucose might have been measurable after overnight fasting or earlier than 24 h post-dose. Previous data in Zucker diabetic fatty (ZDF) rats support this, as dapagliflozin-induced effects on urine glucose were more pronounced at 6 versus 24 h [7]. Han et al. reported that oral dapagliflozin produced a 400-fold increase in urine glucose in normal Sprague–Dawley rats 24 h post-dose, whereas urine glucose was increased only twofold in diabetic ZDF rats [7]. Elvert et al. reported glucose excretion in ZDF rats to be dramatically higher than in lean animals, and the SGLT-2 inhibitor AVE2268 dose-dependently lowered blood glucose, with no effect on glucose excretion [8].

Type 2 diabetes is often associated with chronic kidney disease resulting in gradual renal impairment, which ultimately leads to renal failure. Renal function may affect exenatide pharmacokinetics, due to renal clearance [9], and influence dapagliflozin exposure, metabolism and efficacy [10]. Diabetic *ob*/*ob* mice have dysfunctional kidneys and it has been reported that exenatide exerts renoprotective effects in rodent models [11]; hence, we evaluated renal function at the end of the sub-chronic study. Exenatide + dapagliflozin improved GFR and reduced plasma creatinine, indicating combinatorial benefits on kidney function.

In conclusion, exenatide and dapagliflozin monotherapies significantly improved glycaemia in *ob*/*ob* mice. Exenatide + dapagliflozin regulated basal and postprandial glucose better than either monotherapy, suggesting additive effects. The additive glucoregulatory effects of longer-term combination therapy were less pronounced in this mouse model; however, renal function significantly improved. Thus, combining GLP-1 receptor agonism and SGLT-2 inhibition may be a promising approach in diabetes. Further experimentation to establish optimal doses of exenatide + dapagliflozin to maximize long-term benefits is warranted.

K. Tatarkiewicz, C. Polizzi, C. Villescaz, L. J. D'Souza, Y. Wang, S. Janssen & D. G. Parkes
Amylin Pharmaceuticals, LLC, San Diego, CA, USA

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Conflict of Interest

At the time when the work for this manuscript was performed all authors were employed by Amylin Pharmaceuticals, LLC. All authors held stock in Amylin Pharmaceuticals, LLC.

All authors were involved in study design, data analysis and preparation of the manuscript including decision on content

and editing. C. P. and C. V. performed *in vivo* studies, L. D. developed the method for dapagliflozin synthesis and tested compound solubility and stability and Y. W. and S. J. developed the method and measured dapagliflozin concentration in plasma. K. T. and D. G. P. wrote the manuscript. All authors approved the final manuscript.

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