RESEARCH LETTER

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Reducing hyperglucagonaemia in type 2 diabetes using low-dose glibenclamide: Results of the LEGEND-A pilot study

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1 | INTRODUCTION

Diabetes is a multi-hormonal disorder characterized by insufficient insulin secretion and aberrant glucagon secretion, with fasting hyperglucagonaemia leading to increased hepatic glucose production,[1,2] further exacerbating hyperglycaemia. Low concentrations of sulfonylureas (which close ATP-regulated potassium channels,[3] K_{ATP}) can partially restore appropriate glucose-regulated glucagon secretion in islets isolated from donors with type 2 diabetes (T2D).[4] In this pilot, dose-finding study, we aimed to determine whether and at what dose the sulfonylurea glibenclamide could safely reduce fasting glucagon levels in patients with T2D (diet-controlled or on metformin alone).

2 | METHODS

2.1 | Study participants

Participants were recruited through the Clinical Research Unit (CRU) and the Oxford Biobank databases (Churchill Hospital, Oxford). Inclusion criteria were: >18 years old, diagnosis of T2D (either diet controlled or on metformin alone), body mass index ≤40 kg/m^{2} and glycated haemoglobin between 42 and 80 mmol/mol (6.0% and 9.5%).

Exclusion criteria were: currently pregnant or breastfeeding, estimated glomerular filtration rate <60 ml/min, history of ischaemic heart disease, heart failure or stroke, liver function tests >1.5 times the upper limit of normal range, concomitant use of oral steroids, known malignancy, or receiving a trial drug within 3 months of participation in the current trial.

The study was approved by the South Central – Berkshire B Research Ethics Committee (reference 16/SC/0202). Each patient gave written informed consent.

2.2 | Study design

LEGEND-A (Low-dosE GlibENclamide in Diabetes – part A; NCT02830048) was a 4-week, open-label, non-randomized, dose-titration clinical trial of an oral glibenclamide suspension (GlibenTek[5]). Study visits took place at the CRU in OCDEM (Oxford Centre for Diabetes, Endocrinology and Metabolism), or as home visits when required.

Participants self-administered the oral glibenclamide suspension (0.3-6 mg/day, split dose twice daily), and fasting pre-dose blood samples were taken before each dose increase every 3-4 days (see Figure S1 for trial timeline). Compliance with treatment was checked at each CRU visit, and participants were excluded if they missed >2 doses.

2.3 | Oral glibenclamide suspension

The glibenclamide suspension (GlibenTek[5]) was manufactured by AMMTeK (Paris, France) in two strengths (0.6 and 6 mg/ml).

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The results of this trial were presented at the EASD 2017 meeting (11-15 September 2017 in Lisbon, Portugal) as an oral presentation.

2.4 | Continuous glucose monitoring

The use of masked continuous glucose monitoring (CGM; Freestyle Navigator II; Abbott Diabetes Care) throughout the study was optional. Glucose was measured every 10 min and the result sent to the monitor automatically, but not displayed.

2.5 | Sample handling and biochemical assays

Glucagon samples were collected in pre-chilled tubes containing EDTA and aprotinin, centrifuged at $-4°C$, and stored at $-80°C$ before assaying (ELISA; Mercodia).

Glucose, insulin and C-peptide were assayed using the i200 Immunology Analyzer (Abbott Diagnostics), and pre-dose glibenclamide plasma levels were measured using liquid chromatography tandem-mass spectrometry.

2.6 | Endpoints

The primary endpoint was the dose of glibenclamide that caused a significant decrease in fasting plasma glucagon concentration. The effect on fasting glucose, insulin and C-peptide was also assessed, as well as pre-dose plasma glibenclamide levels. Finally, the overall effect on glycaemic control was assessed using CGM.

2.7 | Sample size calculation and statistical analysis

No human trials have previously used doses of glibenclamide <5 mg in the measurement of glucagon secretion. Sample size calculations based on data from isolated human islets from T2D donors,[4] suggested 15 participants (including 15% dropout) would give the study 80% power to detect a 57% reduction in baseline glucagon (alpha error of 5%). Bonferroni correction was performed for the existence of two subgroups, i.e. 'normal' fasting plasma glucagon, and 'high' (defined in this study as >15 pmol/L; normal range 6-12 pmol/L[6]).

Two-way repeated measures analysis of variance with post-hoc (Holm-Sidak method) multiple comparisons (SigmaPlot 13; Systat Software and GraphPad Prism 7) were used to compare subgroup baseline plasma concentrations of glucagon, glucose, insulin and C-peptide, with those at each dose change. All statistical significances were assessed using a p value of .05 (95% confidence interval).

3 | RESULTS

3.1 | Subgroup inclusion

Participants and research staff were blinded to the baseline values and therefore to subgroup inclusion ('normal' or 'high'), as glucagon measurements were only performed after completion of the trial. Two

TABLE 1 Baseline characteristics of participants

	Normal (n = 12)	High (n = 4)	Adjusted p-value
Age (years)	68 ± 2	52 ± 5	.05
Sex	4 males, 8 females	3 males, 1 female	
BMI (kg/m^2)	30 ± 1	31 ± 3	>.99
Duration of diabetes (years)	5.1 ± 1.8	6.1 ± 2	>.99
HOMA2-B	74 ± 9	63 ± 10	.98
HOMA2-S	56 ± 7	52 ± 6	>.99
HOMA2-IR	2.1 ± 0.3	2.0 ± 0.3	>.99
Taking metformin (participants)	6	3	
Metformin dose (g)	0.5 ± 0.2	1.2 ± 0.4	.75
Baseline blood tests			
Glucagon (pmol/L)	6.2 ± 0.4	25.6 ± 6	<.001
Glucose			
mmol/L	7.3 ± 0.3	7.9 ± 0.4	
mg/dl	131 ± 5	142 ± 8	.95
HbA1c			
mmol/mol	50 ± 2	54 ± 2	
%	6.7 ± 0.2	7.0 ± 0.2	.95
C-peptide (pmol/L)	854 ± 106	799 ± 111	>.99
Insulin (pmol/L)	83 ± 15	74 ± 7	>.99

Note: Data are mean ± SEM. T-tests with Holm-Sidak correction for multiple comparisons used.
Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; HOMA2-B, -S, -IR, homeostatic model assessment of beta-cell function, sensitivity and insulin resistance respectively.

baseline fasting samples were collected (pre-trial visit and trial visit 1), and the mean was used to determine group inclusion for analysis. Details of the variation in baseline blood tests are provided in Figure S2.

3.2 | Patient characteristics

Two participants in the 'normal' group discontinued the intervention at the highest dose because of adverse events (hypoglycaemia),

FIGURE 1 Fasting plasma concentrations of glucagon and glucose, continuous glucose monitoring data showing percentage of time spent hypoglycaemic (glucose <4.0 mmol/L, 72 mg/dl), and pre-dose plasma levels of glibenclamide over the course of the trial. Green bar indicates the normal range of glucagon.[6] Data were analysed using two-way repeated measures ANOVA (comparison made with values at baseline 0 mg in each group), and the graphs show mean ± SEM for each glibenclamide dose along with individual data points (*$p < .05$, **$p < .001$)

therefore an additional participant was recruited as pre-defined in the protocol. Sixteen participants (seven males, nine females) were recruited in total (mean ± SEM): age 64 ± 3 years, body mass index 30.4 ± 1.1, glycated haemoglobin 51 ± 2 mmol/mol (6.8 ± 0.1%) and diabetes duration 5.4 ± 1.5 years. Baseline characteristics of the 'normal' and 'high' group were not significantly different other than fasting glucagon (Table 1).

3.3 | Effect of low-dose glibenclamide

Glibenclamide at a dose of 0.3 mg/day resulted in a 32% reduction from baseline (mean ± SEM: 17.4 ± 2 pmol/L vs. 25.6 ± 2 pmol/L, $p < .05$) in fasting glucagon concentration in the 'high' group (Figure 1). This reduction was not seen at the higher doses, and glibenclamide had no effect on glucagon levels in the 'normal' group.

Baseline fasting insulin and C-peptide were similar between groups (Table 1), and glibenclamide had no effect on fasting levels (Figure S3).

Fasting glucose was unaffected at glibenclamide doses <1.8 mg/day (Figure 1), while CGM from a subset of 12 participants (nine in the 'normal' group, three in the 'high' group) showed a >10-fold increase in the percentage of time spent hypoglycaemic (glucose <4.0 mmol/L, 72 mg/dl) at the highest doses in the 'normal' group (Figure 1). There was no effect on mean glucose (CGM) or time spent hyperglycaemic (glucose >10 mmol/L, 180 mg/dl) (Figure S3).

Pre-dose plasma glibenclamide levels could not be detected at doses ≤1.2 mg/day; however, these rose to (mean ± SEM) 15.3 ± 5.4, 17.2 ± 5 and 29 ± 7.3 ng/ml at 1.8, 3 and 6 mg/day respectively (Figure 1), which were comparable with levels detected by radioimmunoassay in patients with T2D treated with glibenclamide for >1 month.[7]
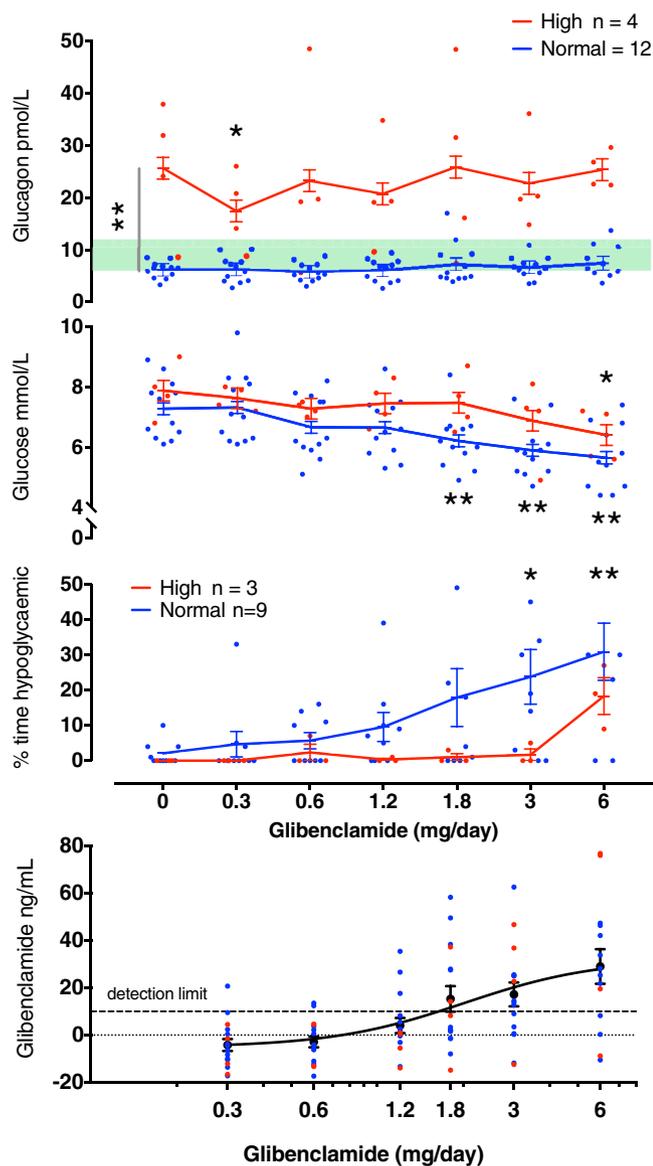
4 | DISCUSSION

We showed that in a subgroup of patients with T2D characterised by inappropriately high fasting glucagon (comparable with levels during induced hypoglycaemia[8]), oral glibenclamide at 0.3 mg/day (<10% the normal starting dose) was able to reduce fasting glucagon by 32% after 3-4 days with no adverse events and without increasing insulin secretion. It is unclear why this effect was not observed at higher concentrations and whether glibenclamide exclusively acts on the glucagon-secreting alpha-cells or also involves the somatostatin-releasing delta-cells of the islet. Somatostatin is a powerful inhibitor of glucagon secretion but available assays do not discriminate between pancreatic and extra-pancreatic somatostatin,[9] and sulfonylurea is a fairly weak stimulus of somatostatin release in isolated human islets.[10] No impact on overall glycaemic control could be shown using CGM but we acknowledge that the trial was not powered to do so. However, doses ≥3 mg/day significantly increased the risk of hypoglycaemia, in keeping with other large-scale studies involving sulfonylureas.[11]

Fasting hyperglucagonaemia was not an entry requirement in this trial, as its prevalence in this population of 'early' (i.e. not on

multiple medications) patients with T2D is not well defined. The impact of low-dose glibenclamide on normal-range fasting glucagon levels was also unknown. In this pilot study, 25% of participants had fasting hyperglucagonaemia; however, other baseline characteristics were not significantly different from the rest of the study population (Table 1). Sulfonylureas have previously been shown to lower glucagon secretion only under hypoglycaemic conditions[12]; our data raise the interesting possibility that this group may have inappropriately high K_{ATP} channel activity under euglycaemic conditions. Studies in isolated islets suggest there is a complex relationship between K_{ATP} channel activity and glucagon secretion[3]; this may resolve the paradox that sulfonylureas were only effective at a single (low) concentration.

This study has some limitations. There was unequal distribution of participants between the 'high'- and the 'normal'-level fasting glucagon groups, because of the unknown prevalence of fasting hyperglucagonaemia and because plasma samples were only tested for glucagon at the end of the trial, to minimise technical variability between assay batches. This could have led to the study being underpowered. The treatment duration was also limited, and only fasting pre-dose blood samples were collected because of limited resources, possibly explaining (together with limited sensitivity of the assay) why plasma glibenclamide levels could not be detected at lower doses. Finally, a statistically significant effect on glucagon levels was achieved only with the lowest of the six tested glibenclamide doses.

Longer follow-up trials are planned, which will also test the effect on postprandial glucagon levels, and the impact on glycaemic control.

5 | CONCLUSION

In conclusion, low-dose glibenclamide safely reduced glucagon levels in a subpopulation of patients with T2D who had fasting hyperglucagonaemia, suggesting that inappropriately increased alpha-cell K_{ATP} activity may be a key component in the metabolic dysregulation of diabetes. Longer follow-up trials are planned, which will test the effect on postprandial glucagon levels, and the impact on glycaemic control.

AUTHOR CONTRIBUTIONS

IS conceived the design of the trial, recruited and screened participants, collected blood samples, analysed the data and drafted the manuscript. RC set-up the mass spectrometry assay along with IS, assayed the plasma samples for glibenclamide and analysed the results. SG and PR provided significant intellectual input, provided financial support for the project and helped draft the manuscript. All authors contributed to the drafting of the manuscript and approved the final version. IS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICT OF INTEREST

IS, RC and PR declare that they have no competing interests. SG reports personal fees from Novo Nordisk A/S, Denmark, outside the submitted work.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14740.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available in the Oxford University Research Archive, with the identifier DOI: 10.5287/bodleian:gJgEVOR64.

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