Glucose Responsive Insulin: An Unprecedented Cleavable Linker Concept

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INTRODUCTION

Achieving the optimal combination of multiple glucose measurements and insulin injections provides a complex daily situation for most insulin-dependent diabetes patients with currently available insulin products. To date, several fast-acting and long-acting insulins have been developed. However, while the pharmacokinetic profiles have been optimized, it has remained an elusive goal for decades to develop a Glucose Responsive Insulin (GRI), which can be responsive to the fluctuations in blood glucose concentrations during the day. Here we present an unprecedented GRI concept.

CONCEPT

The concept is based on the hydrolysis of a cleavable Linker that is covalently bound to insulin. The chemical nature of this central Linker is designed to rapidly release active insulin when glucose rises above euglycemia, and this release of active insulin will increase with increasing blood glucose concentration. The second element of the concept is an albumin-mediated inactivation of the GRI by lipidation. After injection, the GRI will bind to albumin and circulate as a depot, resulting in a slow release of insulin like seen with other basal insulins such as insulin Detemir.

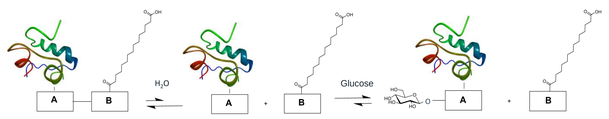


Figure 1 | The concept relies on hydrolysis of a Linker covalently attached to insulin at one end (A) and C-18 fatty acid at the other end (B). Upon hydrolysis, glucose reacts with the free A Linker-moiety resulting in a shift of the equilibrium towards free active insulin. Increasing concentrations of glucose (from normoglycemia to hyperglycemia) drives this shift in equilibrium.

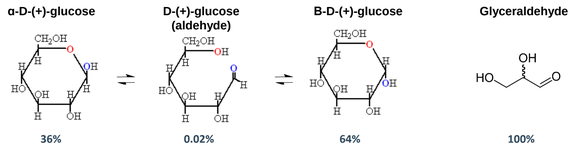


Figure 2 | It is the aldehyde-form of glucose (0.02%) that reacts with the Linker A-moiety. As a crude screening tool, glyceraldehyde (100% aldehyde) can be used to assess the aldehyde-reactivity of the GRIs.

RESULTS

Non-cellular glucose testing of linkers

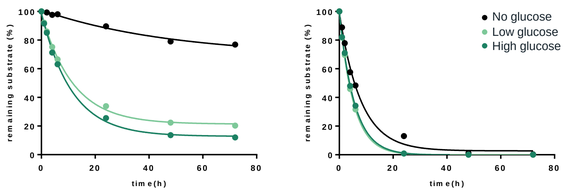


Figure 3 | Linkers were incubated with glucose at different equivalents or without glucose. The remaining substrate was quantified at different time points by UPLC and the half-life was calculated. Examples of a glucose responsive Linker (*left panel*) and non-responsive Linker (*right panel*) are shown.

Cellular glucose testing of a GRI

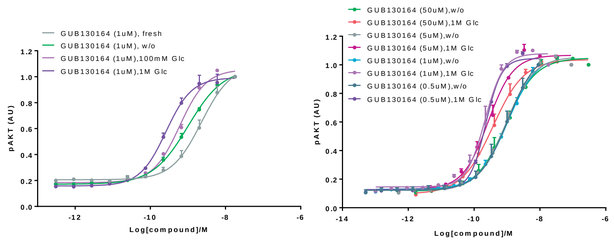


Figure 4 | GUB130164 was incubated at a range of glucose (*left panel*) and/or compounds (*right panel*) concentrations for 24 hours before added to CHO cells overexpressing the human insulin receptor (CHO-IR). Phosphorylated Akt (pAkt) was quantified by ELISA as potency read-out, n=2.

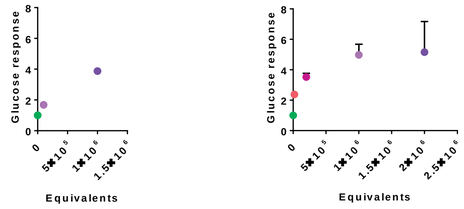
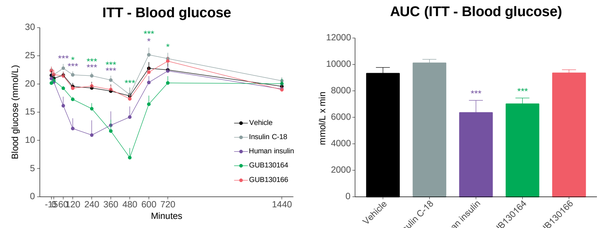


Figure 5 | The glucose response was plotted against the equivalents (glucose/GUB130164) based on the data shown in Figure 4. Similar curves are seen irrespective of varying the glucose concentration (*left panel*) or GRI concentration (*right panel*), indicating that it is the equivalent level that direct the accelerated hydrolysis rate.

In vivo efficacy of GRIs



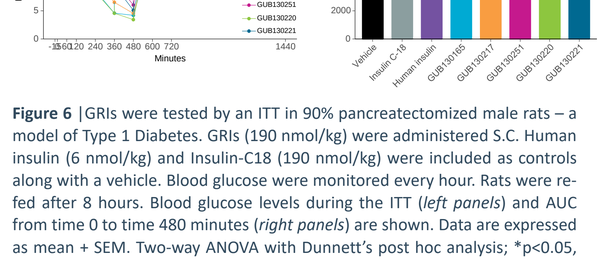


Figure 6 |GRIs were tested by an ITT in 90% pancreatectomized male rats – a model of Type 1 Diabetes. GRIs (190 nmol/kg) were administered S.C. Human insulin (6 nmol/kg) and Insulin-C18 (190 nmol/kg) were included as controls along with a vehicle. Blood glucose were monitored every hour. Rats were re-fed after 8 hours. Blood glucose levels during the ITT (*left panels*) and AUC from time 0 to time 480 minutes (*right panels*) are shown. Data are expressed as mean + SEM. Two-way ANOVA with Dunnett's post hoc analysis; *p<0.05, **p<0.01, ***p<0.001 vs Insulin C-18.

Cellular glyceraldehyde testing of GRIs

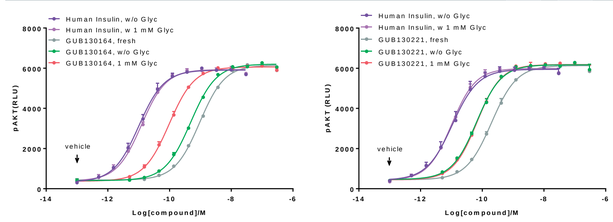
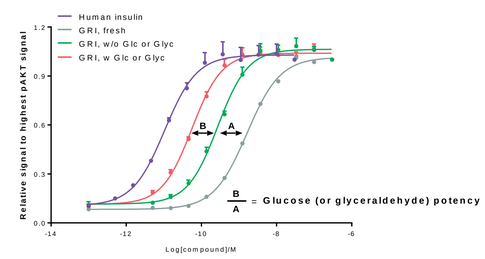


Figure 7 | GRIs were incubated with or without glyceraldehyde for 6 hours before added to CHO-IR cells. pAkt was quantified by ELISA. Human insulin was included as a control, n=2. Examples of an aldehyde-responsive GRI (*left panel*) and non-responsive GRI((*right panel*) are shown.

GRI ranking



GRI	Glucose potency	Glyceraldehyde potency
GUB130164	2.4	4.4
GUB130251	1.8	2.8
GUB130165	1.5	2.3
GUB130166	1.8	1.4
GUB130217	0.9	0.8

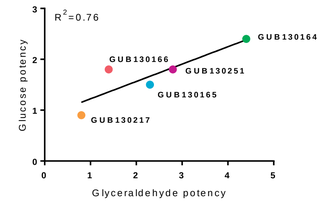


Figure 8 | GRIs can be ranked according to their reactivity defined as the response to either glucose or glyceraldehyde divided with the basal hydrolysis (*top and middle panel*). A good correlation between glucose and glyceraldehyde potency is observed (*lower panel*) validating glyceraldehyde as a suitable *in vitro* screening tool going forward.

CONCLUSIONS

We present a novel linker-based GRI concept and demonstrate the feasibility of a GRI approach that combines the properties of a both basal and bolus insulin. Specifically, we have:

- Demonstrated glucose response on Linkers and full GRIs
- Validated glyceraldehyde as a valuable screening tool *in vitro*
- Addressed the importance of the level of equivalents
- Demonstrated significant and prolonged glucose lowering effects in a Px rat model of Type 1 diabetes