**Long-Acting Glucose-Responsive Insulin with Swift Onset-of-Action**

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**Background and aims.** Long-acting glucose-responsive insulin is anticipated to reduce the frequency of injections by replacing both rapid-acting and long-acting insulin (1). Sequential rapid glucose-responsive insulin release and instant absorption are essential to the swift onset of action (2, 3). Herein, we have developed injectable long-acting glucose-responsive insulin formulations (GRIFs) prepared from glucosamine-modified insulin aspart (ASP-Gn) and phenylboronic acid-modified poly-*ʟ*-lysine (PLL-FPBA). The complex can form a stable GRIF reservoir subcutaneously after injection. Upon food intake, the elevated blood glucose (BG) triggers the release of ASP-Gn, which can be absorbed immediately to downregulate BG back to the normal range.

**Methods.** ASP-Gn was synthesized by conjugating glucosamine to insulin aspart. GRIFs were prepared by mixing PLL-FPBA and ASP-Gn. *In vitro* glucose-responsive insulin release was validated using Coomassie blue. Diabetic mice (1.5 mg/kg) and minipigs (0.06-0.07 mg/kg) received subcutaneous GRIFs injections, with glucose tolerance tests. Biocompatibility was assessed through histopathological analysis.

**Results.** GRIFs exhibited robust glucose-responsive insulin release in vitro, with L2-ASP-G2 demonstrating a glucose stimulation index approaching 400%(ASP: insulin aspart; Gn: the *n* indicated the number of glucosamine moieties in each insulin aspart analog; L1: equal weight of PLL0.4-FPBA0.6 to ASP-Gn; L2: twice the weight of PLL0.4-FPBA0.6 to ASP-Gn). In diabetic mice, L2-ASP-G2 achieved prolonged glycemic control for 13 hours, outperforming the duration achieved by insulin glargine. Diabetic minipigs treated with L2-ASP-G2 achieved normoglycemia within 0.9 hours (compared to 4.5 hours with insulin glargine) and **sustained glycemic control 1.5 times longer**than insulin glargine. No significant fibrous capsule and neutrophil infiltration were observed after administration of L2-ASP-G2.



**Figure 1.** Schematic and glucose-responsive mechanism of the GRIF.

Conclusion/Discussion. Glucose-responsive insulin release, fast onset of action, and prolonged duration all suggest that L2-ASP-G2 is a potential candidate for all-in-one long-acting insulin that can replace both the current long-acting basal insulin and fast-acting insulin.

**References:**

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