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Molecular and Pharmacological Properties of GZR4, a Once -Weekly Insulin Analog

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ABSTRACT

It was reported that the complex, formed between acylated insulin and human serum albumin (HSA), enters an essentially inactive albumin -bound depot, leading to a dramatically decreased potency. This study was performed to develop a more potent once -weekly insulin analog GZR4 and investigate its molecular and pharmacological properties. GZR4 was designed by introducing C22 fatty acid to B29 lysine of insulin backbone (A14E, B16H, B25H, desB30 human insulin) through 12xOEG linker. GZR4 displayed 2-fold increase in HSA binding, while 1.5-fold decrease in IR-A binding, 2.5-fold decrease in IR-B binding compared with GZR64 (an insulin analog with the same structure as insulin Icodec). In the presence of HSA, GZR4 retained IR binding response while GZR64 essentially displayed no binding, the IR phosphorylation activity of GZR4 was approximately 7-fold higher compared with GZR64. Glucose-lowering capability of GZR4 was 3 times higher than that of GZR64 in TIDM STZ treated rats and db/db mice. Further studies demonstrate that the length of the OEG linker between insulin and fatty acid plays an essential role on the potency of the acylated insulins. In the presence of HSA, GZR4 retains partially in vitro bioactivity while GZR64 entered an essentially inactive state. Pharmacological evaluation revealed that GZR4 has the potential to be a novel once-weekly basal insulin.

BACKGROUND

Basal insulin is a key component of insulin therapy in type 1 diabetes (T1D) and remains an indispensable option for glycemic control in type 2 diabetes (T2D) [1].

Once-weekly basal insulins that can maximize treatment adherence, reduce treatment burden without increasing risk of hypoglycemia have been developed in recent years [1, 2]. The most recent once-weekly basal insulins are basal insulin Fc-fusion protein (BIF, LY3209590) developed by Eli Lilly and insulin Icodec developed by Novo Nordisk. BIF is comprised of a single-chain variant of insulin fused to a Fc domain of human IgG2 molecule, which maintains insulin action and attenuates receptor-mediated insulin clearance[3]. Icodec combines amino acid substitutions (A14E, B16H, B25H) with addition of fatty acid to the B-chain of insulin analog, which allows for reduced insulin receptor (IR) binding and reversible albumin binding, further prolonging the half-life of analog[4].

Our study observed that the oligoethylene glycol (OEG) linker connecting fatty diacid with insulin backbone contributes to the stronger albumin binding, lower insulin receptor (IR) binding, and higher hypoglycemic efficacy of acylated insulin. Therefore, in the present study, GZR4, an insulin analog acylated with a 22 fatty acid and contains a long OEG linker, was developed.

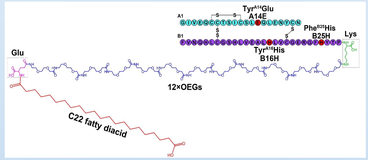
METHODS

1. Preparation of insulin analogs

Insulin backbone (A14E, B16H, B25H, desB30 human insulin) was expressed by recombinant DNA technology. Next, fatty diacids (C22, C20, C18) coupled with different length of OEG linker (12xOEG, 6xOEG, no OEG) was synthesized to obtain side chains. Finally, insulin analogs were prepared by coupling insulin backbone with side chain.
The structure of GZR4, one of these insulin analogs, is shown in Fig 1. It introduced a C22 fatty diacid to B29 lysine through a 12xOEG linker. Other insulin analogs with different lengths of fatty diacids and linker compositions are listed in Table 1.

2. HSA and IR binding assay

The binding affinity of insulin analogs to HSA, hIR-A and hIR-B was determined with surface plasmon resonance (SPR).



▲ Fig 1. Structure of GZR4

Table 1. Insulin substitutions and side chains of insulin analogs

Analog	Insulin substitutions	Side chain
GZR4	A14E, B16H, B25H, desB30	C22-L-γ-Glu-12xOEG
GZR10	A14E, B16H, B25H, desB30	C22-L-γ-Glu-6xOEG
GZR81	A14E, B16H, B25H, desB30	C22-L-γ-Glu-2xOEG
GZR85	A14E, B16H, B25H, desB30	C22-L-γ-Glu
GZR78	A14E, B16H, B25H, desB30	C20-L-γ-Glu-12xOEG
GZR27	A14E, B16H, B25H, desB30	C20-L-γ-Glu-6xOEG
GZR64*	A14E, B16H, B25H, desB30	C20-L-γ-Glu-2xOEG
GZR86	A14E, B16H, B25H, desB30	C20-L-γ-Glu
GZR92	A14E, B16H, B25H, desB30	C18-L-γ-Glu-12xOEG
GZR90	A14E, B16H, B25H, desB30	C18-L-γ-Glu-6xOEG
GZR88	A14E, B16H, B25H, desB30	C18-L-γ-Glu-2xOEG
GZR87	A14E, B16H, B25H, desB30	C18-L-γ-Glu

*The structure of GZR64 is the same as insulin Icodec.

3. Sandwich binding assay

A SPR-based sandwich binding assay was developed on Biacore™ 8K with slightly modifications according to previous studies [5], to investigate the IR binding affinity of insulin analogs in the presence of HSA.

4. In vitro potency assay

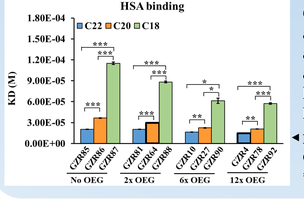
In vitro potency were determined in Chinese Hamster Ovary (CHO) cells overexpressing hIR-B (CHO-IRB) by In-Cell Western (ICW) method.

5. In vivo activity assay

In vivo glucodynamic efficacy was determined in streptozotocin (STZ) induced Type 1 Diabetes Mellitus (T1DM) rat model and db/db mice.

RESULTS

1. HSA binding affinity increased with the increase length of fatty diacid and OEG linker



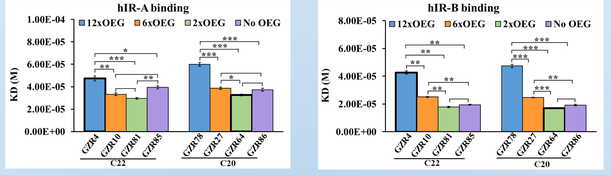
GZR4 showed a 2-fold higher HSA binding affinity than GZR64. Further study for insulin analogs with different lengths of fatty diacids and linker compositions demonstrated that the longer fatty diacid and OEG linker, the higher HSA binding affinity.

◀ Fig 2. HSA binding affinity of insulin analogs with different lengths of fatty diacids and OEG linker

*p<0.05, **p<0.01, ***p<0.001.

2. IR binding affinity decreased when the number of OEG linker increased to 12

GZR4 displayed a 1.5-fold lower hIR-A binding affinities compared to GZR64, and a 2.5-fold lower hIR-B binding affinities. Further study demonstrated that for insulin analogs with same fatty diacids (C22 or C20), the hIR-A and hIR-B binding affinities were significantly decreased when the number of OEG linker increased to 12.



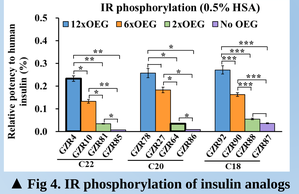
▲ Fig 3. IR binding affinity of insulin analogs with different lengths of fatty diacids and OEG linker

*p<0.05, **p<0.01, ***p<0.001.

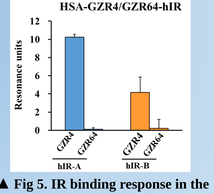
3. GZR4 retained in vitro biological activity in the presence of HSA

Compared with GZR64, the IR phosphorylation activity of GZR4 was approximately 7-fold higher in the presence of 0.5% HSA . Further study demonstrated that IR phosphorylation activity of insulin analogs decreased as the truncation of OEG linker (Fig 4).

In the presence of HSA, GZR4 retained insulin receptor binding response with 10.2 ± 1.3 RU while GZR64 essentially displayed no binding signals (Fig 5).



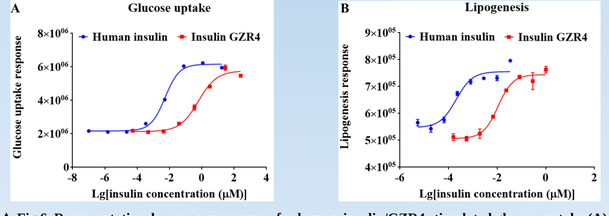
▲ Fig 4. IR phosphorylation of insulin analogs in the presence of 0.5% HSA



▲ Fig 5. IR binding response in the presence of HSA

*p<0.05, **p<0.01, ***p<0.001.

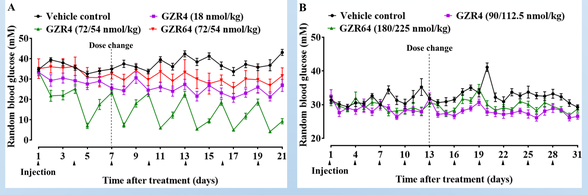
GZR4 stimulated glucose uptake and lipogenesis in a similar dose-dependent manner as human insulin, indicating that GZR4 retained the biological properties of human insulin.



▲ Fig 6. Representative dose-response curves for human insulin/GZR4 stimulated glucose uptake (A) and lipogenesis (B) in 3T3-L1 MBX adipocytes

4. In vivo glucodynamic potency of GZR4 was higher than that of GZR64

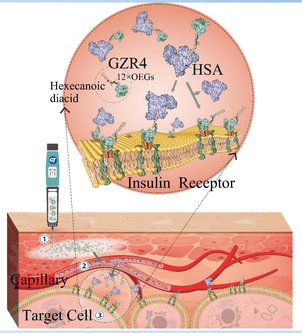
In T1DM STZ rats, GZR4 dosed at 18 nmol/kg exhibited comparable glucodynamic efficacy to that of GZR64 dosed at 72/54 nmol/kg, indicating that GZR4 is 3 time more potent than GZR64. In db/db mice, GZR4 dosed at 90/112.5 nmol/kg exhibited comparable glucodynamic efficacy to that of GZR64 dosed at 180/225 nmol/kg, indicating that GZR4 is 2 time more potent than GZR64.



▲ Fig 7. Random blood glucose in T1DM STZ rats (A) and db/db mice (B)

CONCLUSION

GZR4 displayed higher HSA binding affinity and lower hIR-A/hIR-B binding affinity than GZR64. Further study demonstrated that the length of OEG linker between insulin and fatty diacids plays an essential role on the HSA binding affinity, hIR-A/hIR-B binding affinity, and potency of acylated insulins. In the presence of HSA, GZR4 retained partially in vitro bioactivity. Accordingly, GZR4 showed higher in vivo glycemic control effects than GZR64. These findings indicate that GZR4 has the potential to be a novel basal insulin suitable for once-weekly dosing.



◀ Fig 8. A hypothesized scheme for mechanism of action of GZR4

① After injection, GZR4 hexamers dissociate into monomers, bind to albumin in the depot, re-dissociate into GZR4 monomers, and transfer to capillaries.
② In capillaries, GZR4 binds to plasma albumin to form a GZR4-albumin complex, which slowly dissociates into GZR4 and passes through the endothelial cell layer of capillaries into the interstitium.
③ In the interstitium, GZR4 binds to interstitial albumin to form a GZR4-albumin complex. Part of the complex dissociates into GZR4 and then binds to insulin receptor on the surface of target cells, while the other part binds to insulin receptor directly.

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