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Species-Specific Effects of Neuregulin-1 β (Cimaglermin alfa) on Glucose Handling in Animal Models and Humans with Heart Failure

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Abstract

Neuregulin-1 β is a member of the neuregulin family of growth factors and is critically important for normal development and functioning of the heart and brain. A recombinant version of neuregulin-1 β , cimaglermin alfa (also known as glial growth factor 2 or GGF2) is being investigated as a possible therapy for heart failure. Previous studies suggest that neuregulin-1 β stimulation of skeletal muscle increases glucose uptake and, specifically, sufficient doses of cimaglermin alfa acutely produce hypoglycemia in pigs. Since acute hypoglycemia could be a safety concern, blood glucose changes in the above pig study were further investigated. In addition, basal glucose and glucose disposal were investigated in mice. Finally, as part of standard clinical chemistry profiling in a single ascending-dose human safety study, blood glucose levels were evaluated in patients with heart failure after cimaglermin alfa treatment. A single intravenous injection of cimaglermin alfa at doses of 0.8 mg/kg and 2.6 mg/kg in mice resulted in a transient reduction of blood glucose concentrations of approximately 20% and 34%, respectively, at 2 hours after the treatment compared to pre-treatment levels. Similar results were observed in diabetic mice. Treatment with cimaglermin alfa also increased blood glucose disposal following oral challenge in mice. However, no significant alterations in blood glucose concentrations were found in human heart failure patients at 0.5 and 2 hours after treatment with cimaglermin alfa over an equivalent human dose range, based on body surface area. Taken together, these data indicate strong species differences in blood glucose handling after cimaglermin alfa treatment, and particularly do not indicate that this phenomenon should affect human subjects.

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Conflict of Interest

JHC and AM have no conflict of interest.

Keywords

neuregulins; glucose handling; animal models; humans

Introduction

Neuregulins (NRGs) belong to the epidermal growth factor (EGF) family of proteins. They are produced as multiple splice variants from four structurally related genes (NRG1–4). NRG1 has been the most characterized of the neuregulin variants and its effects have been studied in a variety of tissues. NRG 1 binds to tyrosine kinase receptors ErbB3 and ErbB4, which form homodimers or ErbB2 heterodimers. Dimerization induces ErbB receptor phosphorylation, activating a number of downstream intracellular signaling proteins via multiple mechanisms including PI3K-Akt and MAPK/ERK pathways (Carraway and Cantley, 1994, Yarden and Sliwkowski, 2001, Gumà et al, 2010). These signaling cascades affect transcription of a number of gene products that are essential for growth, differentiation and survival of the heart and nervous system (Falls, 2003).

NRG 1 β is expressed in the coronary microvascular endothelium and endocardium (Lemmens et al., 2006; Russell et al., 1999; Zhao et al., 1998). Cardioprotective effects produced by NRG 1 β have been observed in *in vitro* studies (Sawyer, et al., 2002; Fukuzawa, et al., 2003). In addition, NRG 1 β (a fragment encoding the EGF-like region of NRG-1 and encompassing residues Ser177 to Glu237) was reported to improve cardiac function and survival in animal models of ischemic, dilated and viral cardiomyopathy (Liu, et al., 2006). Cimaglermin alfa (also known as glial growth factor 2 or GGF2) is a soluble full-length form of secreted neuregulin 1 β 3. Repeated treatment with cimaglermin alfa in rats (weekly or once every two weeks) and pigs (twice weekly) was found to improve cardiac function after myocardial infarction (MI, Hill, et al., 2013, Galindo, et al., 2014, Parry, et al., 2017), and also to prevent adverse remodeling post-MI in pigs (Galindo, et al., 2014). Cimaglermin alfa has been evaluated in people with the chronic heart failure (Lenihan, et al., 2016). In addition to effects in heart failure, cimaglermin alfa and other neuregulins have shown potential as therapeutic agents in stroke (Iaci, et al., 2010 and 2016), Parkinson's disease (Zhang et al., 2004, Carlsson et al., 2011), peripheral nerve injury (Burnett et al., 2015), and spinal cord injury (Whittaker, et al., 2012).

In addition to playing an important role in cardiac function, NRG 1 β is involved in the regulation of glucose metabolism (Gumà, et al., 2010; Ennequin et al., 2015; Caillaud, et al., 2016). NRG 1 β can influence glucose homeostasis through effects on the translocation of glucose transporters in rat L6E9 myoblasts (Suárez et al, 2001). In addition, liver ErbB3 expression is reduced after insulin treatment in two animal models of insulin deficiency (type I diabetes and fasting), suggesting an interaction between insulin and the NRG 1 β / ErbB pathway in maintenance of glucose homeostasis (Carver, et al., 1997). Thus, NRG 1 β may act in a paracrine, juxtacrine and/or endocrine signaling manner to increase glucose uptake from the circulation. Although the role of neuregulins in glucose metabolism has recently attracted attention, its effects on blood glucose handling across species, and humans in particular, are not well known.

During early pharmacodynamic testing of cimaglermin alfa in pigs with heart failure induced by surgical myocardial infarction, it was noted that cimaglermin alfa substantially reduced blood glucose shortly after infusion. This finding was briefly discussed in our previous publication reporting therapeutic effect of cimaglermin alfa on improvement of cardiac function after heart failure (Galindo et al., 2014). Given this finding of hypoglycemia, we have subsequently analyzed in detail the blood glucose data from the previous pig study. We also analyzed blood glucose data from a clinical study examining the safety and tolerability of cimaglermin alfa in patients with heart failure and conducted additional studies in mice to specifically evaluate glucose handling after treatment with cimaglermin alfa in that species. The data from all of the above studies were compiled to compare the effects of intravenous cimaglermin alfa administration on blood glucose levels in pigs, mice and humans. Our findings show that there are major species differences in the acute effects of cimaglermin alfa on basal blood glucose levels. The potential mechanisms for these differences and their implications for the development of cimaglermin alfa as a therapy for human disease are discussed.

Methods

Compounds

Cimaglermin alfa was produced and purified under GMP conditions at CMC Biologics (Bothell, WA) for clinical investigation (Lenihan, et al, 2016) or under GMP-like conditions at Acorda Therapeutics, Inc. (Ardsley, NY) for animal studies. The vehicle (placebo) consisted of 20 mM histidine, 100 mM sodium sulfate, 100 mM L-arginine, and 1% mannitol (w/v), at pH 6.5.

Subjects and procedures

Pigs—All procedures in pigs were approved by the Vanderbilt IACUC and were conducted according to Association for the Accreditation of Laboratory Animal Care (AAALAC) international standards, and the Guide for the Care and Use of Laboratory Animals (Version 2011), the Animal Welfare Act and the 2013 AVMA Guidelines for the Euthanasia of Animals. Male Yorkshire pigs were anesthetized with isoflurane and underwent endovascular occlusion of the left anterior descending (LAD) coronary artery to induce MI (Galindo et al., 2014). At 7 days post-MI, an indwelling catheter was implanted in an ear vein under general anesthesia. From 7 to 35 days post-MI, cimaglermin alfa, at a dose of either 0.67 or 2 mg/kg was intravenously administered by way of IV extension sets over 15 minutes for a total 8 doses (twice per week, every 3 to 4 days). Blood samples were collected at intervals of 5 or 10 minutes for the initial 1 hour after administration of the last dose for determination of blood glucose levels.

Mice—All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Acorda Therapeutics Inc. and conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Eight-week old male C57 BL/6J (normal mice) and B6.BKS(D)-Lepr^{db}/J (db/db, diabetic mice) were ordered from the Jackson Laboratory (JAX, Bar Harbor, Maine). Animals were housed in cages containing Diamond Dri Bedding under diurnal lighting conditions and allowed free

access to food (Harlan Teklad Global 14% protein rodent maintenance diet) and water *ad libitum*. Mice were acclimated for at least five days after delivery. Mice did not receive surgical-induced MI's. Mice were assigned to groups receiving cimagermin alfa (0.8 or 2.6 mg/kg) or vehicle solutions injected as a bolus via tail vein. The doses were selected based on previous studies, which showed that cimagermin alfa promotes improvement in left ventricular function in rats following MI-induced heart failure (Parry, et al., 2017).

Humans: The subjects in an IRB-approved, randomized, placebo-controlled, Phase 1, single, ascending dose clinical trial (NCT01944683) were men or women 18–75 years of age with NYHA Class II–III heart failure having reduced left ventricular ejection fractions between 10–40% and on optimized medical therapy. The clinical trial was conducted at 9 sites in the United States. Study drug or the placebo was administered as a 100 ml infusion delivered over 20 minutes. Six subjects received placebo, 5 subjects, each, received cimagermin alfa at 0.06 mg/kg, 0.19 mg/kg and 0.38 mg/kg. Blood samples were collected immediately before, at 0.5 and 2 hours after cimagermin alfa treatment for measurement of blood glucose concentrations.

Blood Glucose Determination

In mice, blood glucose concentrations were measured from blood obtained via the tail vein, using a handheld glucometer (OneTouch Ultra2, LifeScan). In pigs, blood samples were analyzed for blood glucose concentrations by a CRO lab (Antech Diagnostics, Irvine, CA). In humans, blood glucose concentrations were determined through standard clinical chemistry testing at a central laboratory (Pharmaceutical Product Development, PPD®, Wilmington, NC).

Oral Glucose Tolerance Test (OGTT) in Mice

OGTT is a widely-used test to assess blood glucose handling (Ennequin, et al, 2015, López-Soldado, et al, 2016) and was evaluated only in mice in the present study in order to characterize the effects of cimagermin alfa on glucose disposal. Mice were fasted for approximately 6 hours in the morning. Cimagermin alfa was administered intravenously at doses of either 0.8 or 2.6 mg/kg 4 hours after initiation of the 6 h fast. The animals received a glucose (1 or 2 g/kg body weight for *db/db* or C57 BL/6J mice, respectively) challenge by gastric gavage with 20% or 40% glucose solution (5 mL/kg) for *db/db* or C57 BL/6J mice, respectively, at 2 hours after cimagermin alfa treatment. Five microliter blood samples were taken immediately before, at 30, 60 and 120 minutes after the oral glucose challenge to determine blood glucose levels. Areas under the blood glucose concentration-time curve over 120 minutes were calculated as an index of glucose disposal following vehicle or cimagermin alfa treatment.

Insulin and Glucagon Assay

Blood in pigs treated with cimagermin alfa at 0.67 mg/kg was sampled before and at 50–60 minutes after the treatment. The blood was centrifuged for serum collection and kept in –80°C. Immunoassays were performed on serum for insulin (ALPCO, 80-INSPO-E01 Insulin ELISA) and glucagon (Phoenix Pharma EK-028-02 Porcine Glucagon ELISA) according to manufacturer's instructions.

Statistical Analysis

Group mean and standard errors of the mean were calculated for each group. Group means were compared using one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's multiple comparison tests. Whenever two variables (e.g. time and treatment) were involved, means were compared using a two-way ANOVA followed by a Tukey's multiple comparisons test or Bonferroni post-test. The group differences in insulin and glucagon were analyzed with paired Student *t*-test. Values of *p* less than 0.05 were considered statistically significant, analyzed with GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA).

Results

Effects of cimaglermin alfa on blood glucose, insulin and glucagon concentrations in pigs after myocardial infarction

As reported previously (Galindo, et al., 2014), pigs (n=2) at days 7–35 post-MI were treated with cimaglermin alfa intravenously at dose of 2 mg/kg, twice per week for a total 8 infusions. Within 15 minutes of the second cimaglermin alfa treatment, the animals developed lethargy with vomiting and convulsions. In response to these initial observations emergency serum chemistry was obtained and revealed profound hypoglycemia (20 mg/dL serum glucose level, normal range of 48–145 mg/dL) without abnormalities in other electrolytes or other parameters. The hypoglycemic effect lasted for 60–90 minutes and the animals recovered completely between doses. Subsequently 50% dextrose solution (50–100 mL per animal) was intravenously injected as a bolus following each cimaglermin alfa administration to reduce the severity of the lethargy and to prevent vomiting and convulsion. With these prophylactic measures, the 2 pigs completed the full course of cimaglermin alfa treatment of 8 doses of 2 mg/kg. Blood glucose levels were reassessed following the last dose of cimaglermin alfa, again showing hypoglycemia (Fig. 1A).

Due to the hypoglycemic-associated lethargy following cimaglermin alfa treatment, the dose of cimaglermin alfa was thereafter reduced from 2 mg/kg to 0.67 mg/kg for the other pigs with MI (n=7) in the study. In addition, supplemental 50% dextrose (50–100 mL per pig) was administered intravenously as a bolus during the post-cimaglermin alfa infusion period. Animals tolerated this low dose of 0.67 mg/kg. However, the hypoglycemia remained, with a significant reduction in blood glucose levels from 30 to 60 minutes after cimaglermin alfa administration (Fig. 1B). There was no significant difference in serum insulin and glucagon concentrations between baseline and at 50–60 minutes after treatment with cimaglermin alfa at 0.67 mg/kg (Table 1).

Effects of cimaglermin alfa on basal blood glucose concentrations and glucose tolerance in normal and diabetic mice

Based on the glucose findings in pigs, the effect of cimaglermin alfa on glucose handling was examined in mice. A single intravenously injection of cimaglermin alfa at doses of 0.8 and 2.6 mg/kg resulted in significant reduction of fasting blood glucose levels by 31% and 42%, respectively, as compared to the vehicle treated group at 2 hours after the treatment in normal C57 BL/6J mice. When compared to the pretreatment levels, administration of cimaglermin alfa at doses of 0.8 and 2.6 mg/kg led to significant decreases 20% and 34% in

blood glucose levels, respectively, while injection of vehicle solution was associated with a minor but significant increase in blood glucose concentrations relative to pretreatment, which might be due to the effect of stress from handling for *iv* injection (Fig. 2A). No lethargy was observed in mice after *iv* injection of cimagermin alfa. Similar results were observed in diabetic db/db mice (Fig. 2B). The effects of cimagermin alfa on blood glucose levels at other time points were tested in another cohort of C57 Black/6J mice. Thirty minutes after intravenous injection, cimagermin alfa treatment of 0.8 and 2.6 mg/kg significantly reduced blood glucose concentrations as compared to their individual pretreatment levels and compared to the vehicle-treated group. At 4 hours after the treatment, while blood glucose in the mice treated with cimagermin alfa at dose of 2.6 mg/kg remained significantly lower than the vehicle-treatment group, blood glucose in mice treated with a dose of 0.8 mg/kg returned to the level in vehicle-treated animals (Fig. 3). Blood glucose levels returned to baseline values at 24 and 48 hours after the injection, indicating that the effect of reduction in blood glucose by cimagermin alfa was acute and transient (Fig. 2A).

To evaluate the effects of cimagermin alfa on glucose disposal, an OGTT was conducted two hours after intravenous injection of cimagermin alfa in a different cohort of C57 BL/6J and db/db mice. The basal blood glucose levels prior to oral glucose administration were significantly lower in cimagermin alfa-treated mice (130 ± 7 and 109 ± 5 mg/dL at doses of 0.8 and 2.6 mg/kg, respectively, $n=12$ each) than that in vehicle-treated animals (189 ± 4 mg/dL, $n=12$), confirming the activity of cimagermin alfa on blood glucose reduction. Following oral glucose administration, blood glucose levels increased rapidly and then returned gradually to baseline values over 120 minutes in vehicle-treated animals. A similar trend in blood glucose levels occurred following oral glucose administration in cimagermin alfa-treated animals; however, glucose disposal following the oral glucose administration was increased by cimagermin alfa treatment, as evidenced by the integrated glucose concentration-time response (area under the curve, AUC), an index of overall glucose disposal calculated over the 120 min post-glucose administration observation period, showed that cimagermin alfa treatment produced a significant and dose-dependent decrease in AUC (increased glucose disposal) *vs.* the vehicle treatment (Fig. 4).

The effect of once every two week intravenous cimagermin alfa administration on blood glucose was examined, since this is a regimen known to produce a sustained improvement in left ventricular function in a rat model of heart failure (Parry, et al, 2017). To avoid the expected acute effects of cimagermin alfa on blood glucose, fasting blood glucose levels were measured 24 h following each treatment (a total of 3 treatments). In contrast to the blood glucose reduction found acutely (0.5 and 2 hours) after cimagermin alfa injection, no significant difference in blood glucose levels was detected at 24 hours following any of the cimagermin alfa doses as compared to the vehicle-treated group, suggesting that repeated, infrequent dosing of cimagermin alfa does not promote sustained blood glucose lowering (Fig. 5).

Effects of cimaglermin alfa on blood glucose concentrations in humans with heart failure

In light of the initial findings in pigs, the effects of cimaglermin alfa on blood glucose levels were carefully monitored in Study NCT01944683 which was examining the safety and tolerability of cimaglermin alfa in patients with heart failure. Cimaglermin alfa treatment produced no significant alterations in blood glucose concentrations or symptoms typically associated with hypoglycemia over 2 hours following intravenous infusion at doses up to 0.378 mg/kg in patients with heart failure (Fig. 6). Cimaglermin alfa treatment did not produce statistically significant changes in blood glucose levels in male or female patients (Table 2).

Discussion

Blood glucose regulation is tightly controlled in mammals and appears to be affected by a number of endocrine and paracrine factors including NRG 1 β . NRG 1 β has been found to be involved in blood glucose handling in L6E9 rat skeletal muscle cell culture (Suárez et al, 2001). Signaling through ErbB3 activation of PI3K/protein kinase B/Akt pathway, NRG 1 β increases translocation of glucose transporters, including GLUT4, as well as expression of the transporters in skeletal muscle cells *in vitro*, therefore stimulating glucose uptake (Suárez et al, 2001; Cantó, et al, 2004). In *in vivo* studies, a single injection (i.p.) of NRG 1 β was found to reduce blood glucose concentrations over 30–60 minutes and to increase blood glucose disposal over 60–90 minutes after treatment, as compared to vehicle treatment in diabetic mice. Glucose disposal was also increased after the NRG 1 β administration in diabetic mice (Ennequin et al, 2015). Similarly, NRG 1 β was found to promote glucose disposal in normal and diabetic rats (López-Soldado et al, 2016). Consistent with these previous findings, the present study revealed that cimaglermin alfa, a full-length NRG 1 β variant (Iaci et al, 2010; Parry et al, 2017) reduces blood glucose concentrations in pigs and mice and increases blood glucose disposal in mice. These data are consistent with and further support the previous reports of neuregulin enhancing glucose uptake and clearly indicate that systemic administration of pharmacologic doses of NRG 1 β affect blood glucose handling in mice and pigs.

The glucose lowering effect of cimaglermin alfa appears to be related to handling glucose at the level of the glucose transporter. In an *in vitro* study using L6E9 myotubes, Cantó and colleagues (Cantó et al, 2004) reported that NRGs directly stimulated translocation of glucose transporter by activation of a pathway that required PI3K, PDK1 (3-phosphoinositide dependent protein kinase-1) and PKC ζ (protein kinase C, zeta). NRG and insulin produce additive effects on glucose uptake in muscle cells, indicating that NRG coordinates with insulin in regulation of blood glucose (Suárez et al, 2001). It is unlikely that the acute effects of cimaglermin alfa on blood glucose were mediated by acute insulin secretion since serum analyzed from cimaglermin alfa-treated pigs did not show a change in serum insulin. There was also no change in serum glucagon, another critical regulator of glucose (Table 1).

One of the major findings in the present study was a dramatic species-related sensitivity to the acute effect of pharmacologically-relevant dose levels of cimaglermin alfa on basal blood glucose levels in pigs, mice and humans. While only a relatively mild and transient decrease

in blood glucose was elicited by cimaglermin alfa treatment in mice, a severe and transient hypoglycemia following a transient rise in blood glucose (likely due to injection stress) accompanying a lethargic condition was observed in pigs following cimaglermin alfa administration. Cimaglermin alfa infusion had no clinically significant effects on blood glucose concentrations in human patients with heart failure, which is consistent with the findings in a previous clinical study (NCT01258387) that no signs or symptoms of hypoglycemia were observed in heart failure patients treated with higher dose (up to 1.512 mg/kg) of cimaglermin alfa (Lenihan, et al, 2016). These results indicate differential effects of cimaglermin alfa on glucose handling as a function of species. While cimaglermin alfa enhances glucose uptake in pigs and mice, the effects in species are very different and nearly unmeasurable in humans.

The clearance of a drug can vary widely across species, affected by blood flow, metabolic rate, renal and hepatic function and other parameters that are related to body surface area (Friereich et al., 1966). Allometric scaling of drug dosages as a function of body surface area is a well-accepted method for estimating species-equivalent dose levels across species. The US Food and Drug Administration established a guidance for conversion of dose among animals and humans (USFDA 2005). Based on this guidance, the dose of 0.8 mg/kg in mice that elicited significant blood glucose reduction in the present study is equivalent to a dose of 0.065 mg/kg in humans. In the current study, a human cimaglermin alfa dose of 0.378 mg/kg (6-fold greater) had no significant effects on blood glucose in humans with heart failure. The dose of 0.67 mg/kg in pigs, at which cimaglermin alfa treatment resulted in a severe hypoglycemia in pigs, is equivalent to a dose of 0.634 mg/kg in humans. When cimaglermin alfa was tested at a dose of 0.378 mg/kg, no blood glucose reduction was observed in humans. These results suggest that the effects of cimaglermin alfa administered in a pharmacologic dose range on blood glucose are highly species dependent.

Skeletal muscle and adipose tissues are the major depots of glucose disposal. A high ratio of skeletal muscle and adipose tissue relative to body weight in pigs likely provides for a large tissue mass capable of removing glucose from circulation following cimaglermin alfa treatment. While GLUT4 is the primary insulin-sensitive glucose transporter in skeletal muscle and adipocytes (James et al, 1988), both GLUT4 (insulin-regulated) and GLUT2 are expressed in both skeletal muscle and adipose in multiple pig breeds (Liang et al., 2015). GLUT1 is also expressed widely in peripheral tissues, but plays a primary role in basal glucose transport (Assimakopoulos-Jeannet et al., 1991). Given that pig breeding selects for skeletal muscle and fat development, the sheer mass of skeletal muscle and adipose tissue in pigs could account for the exaggerated glucose lowering effect of cimaglermin alfa, possibly through neuregulin-enhanced plasma membrane translocation of GLUT4.

Kidneys make significant contributions to maintaining glucose homeostasis. When circulated in the kidneys, blood glucose is filtered in glomeruli and then reabsorbed through glucose transporters within the proximal tubules, rendering the urine virtually glucose-free in the normal condition. Abnormalities in this process can result in alterations in both blood and urine glucose concentrations. Given the acute effects of cimaglermin alfa on blood glucose reduction in pigs and mice observed in the current study, it merits future

investigation whether intravenous infusion of cimaglermin alfa affects blood glucose regulation via alterations in renal glucose handling.

Since both neuregulin and insulin affect glucose levels in pigs and mice, it is interesting to compare the relative sensitivity of blood glucose disposal of the both species to insulin. While a dose of 0.03 U/kg of insulin in pigs is sufficient to induce hypoglycemia (Bonneau, 1993), a more than 10-fold higher dose (0.5 U/kg) of insulin is required in mice to elicit a similar blood glucose lowering effect (Heikkinen et al, 2007), suggesting pigs are more sensitive than mice in response to insulin challenge. Interestingly, the plasma clearance of human insulin is similar across pigs, dogs and rats (Plum et al., 2000). As such, the effects of systemically administered insulin appear to be related to pharmacodynamic sensitivity differences across species. Our results suggest that pharmacodynamic sensitivity differences in the acute effects on glucose uptake exist for neuregulins as well.

It is notable that one of the components in the vehicle (placebo) solution used in the current study is L-arginine. Recent studies indicated that long-term oral administration of L-arginine increases insulin sensitivity in type II diabetic patients (Piatti et al, 2001; Lucotti et al, 2006). This pathway might confound the cimaglermin alfa effect on blood glucose reduction. An effect of arginine in vehicle solution on glucose reduction is unlikely, however, since the treatment of cimaglermin alfa and vehicle solution was acute in the current study. No blood glucose reduction was observed in mice or humans after injection of L-arginine containing vehicle solution.

Species-specific differences need to be considered when translating drug findings from bench to bedside. Despite similarities, glucose handling in humans differs from other species (Chandrasekera and Pippin, 2014) at every level from gene/protein expression, cellular signaling, tissue and organ to the whole organism. Specifically, GLUT4 (the key modulator of insulin-sensitive glucose uptake) expression levels in skeletal muscle of pigs are higher compared to that in rats (Duehlmeier et al, 2007), which might partially explain the severe hypoglycemia in pigs after cimaglermin alfa treatment. This notion of species-specific difference in glucose regulation is supported by our study findings that human blood glucose responses to similar cimaglermin alfa dose levels as a function of body surface area differs from mice and pigs. These results underscore the importance of understanding relative species effects in evaluation of drug safety on glucose homeostasis.

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Highlights

- Cimaglermin alfa acutely reduced blood glucose levels in pigs, less so in mice
- Cimaglermin alfa did not reduce blood glucose levels in heart failure patients
- Effects of cimaglermin alfa on blood glucose regulation are species-dependent

Figure 1.

Effects of cimagermin alfa on blood glucose concentrations in pigs with heart failure. **(A)** At dose of 2 mg/kg, cimagermin alfa treatment resulted in a severe hypoglycemia (<50 mg/dL of blood glucose) acutely after the drug infusion in 2 pigs with heart failure. **(B)** At dose of 0.67 mg/kg, cimagermin alfa infusion induced a gradual and significant reduction in blood glucose. n=7. Data are expressed as group mean±SEM. The group means at baseline and after cimagermin alfa treatment was compared statistically by one-way ANOVA followed by Dunnett's multiple comparisons test. Asterisks denote values of $p<0.05$ vs.

baseline at time “0” minute. The initial rise in blood glucose levels might be caused by stress from handling for injection.

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Figure 2.

Effects of a single injection of cimaglermin alfa on basal blood glucose concentrations in normal (A) and diabetic (B) mice. Solutions of cimaglermin alfa at doses of 0.8 mg/kg (n=12), 2.6 mg/kg (n=12) or vehicle (n=12) were injected as a bolus via the tail vein in normal (C57 BL/6J) and diabetic (db/db) mice separately. The experimental course of drug administration and blood glucose assay is explained in the schematic (top panel). Significant reduction in blood glucose was present at 2 hours after cimaglermin alfa injection in C57 BL/6J and db/db mice as compared to vehicle-treated group. However, this change was absent when examined at 24 and 48 hours after the drug treatment in C57BL/6J mice. Data are expressed as group mean $\pm$ SEM. Group means were compared statistically with two-way ANOVA followed by Tukey's multiple comparisons test. Asterisks of ** and ++ denote values of $p<0.01$ vs. group of Vehicle and as compared to the pretreatment levels, respectively. Of note, blood glucose concentrations in all groups (including Vehicle group) after *iv* injection were significantly different from their corresponding baseline levels before the injection in C57 BL/6J mice. This might be contributed to the effect of stress from manipulation for *iv* injection and/or the effect of timing at measurement for baseline (8:00) and after administration (14:00) (See the schematic).

Figure 3.

Time course of changes in basal blood glucose concentrations after a single injection of cimaglermin alfa in C57 BL/6J mice. Solutions of cimaglermin alfa at doses of 0.8 mg/kg (n=8), 2.6 mg/kg (n=8) or vehicle (n=8) were injected as a bolus via the tail vein at 2 hours after removal of the food. Data are expressed as group mean $\pm$ SEM. Group means were compared statistically with two-way ANOVA followed by Tukey's multiple comparisons test. Single and double asterisks (* and **) denote values of $p<0.05$ and $p<0.01$ vs. time "0", respectively. Marks of ++ and ^ represent values of $p<0.01$ and $p<0.05$ vs vehicle group and cimaglermin alfa 0.8 mg/kg group, respectively.

Figure 4.

Effects of a single injection of cimagermin alfa on glucose tolerance in normal and diabetic mice. Disposal of blood glucose after cimagermin alfa treatment was evaluated with oral glucose tolerance test (OGTT) in C57 BL/6J and db/db mice. Solutions of cimagermin alfa at doses of 0.8 mg/kg (n=8), 2 mg/kg (n=8) or vehicle (n=8) were injected as a bolus via the tail vein at 2 hours prior to oral glucose challenge (top panel). Blood glucose concentrations were measured immediately, 30, 60 and 120 minutes after the oral glucose administration (top panel). Data are expressed as group mean $\pm$ SEM. Group means were compared statistically with oneway ANOVA followed by Tukey's post-test. Asterisks of * and ^ denote values of $p < 0.05$ vs. group of Vehicle and group of cimagermin alfa at 0.8 mg/kg, respectively.

Figure 5.

Effects of multiple injections of cimagermin alfa on basal blood glucose concentrations in normal mice. Solutions of cimagermin alfa at doses of 0.8, 2 mg/kg or vehicle were injected as a bolus via the tail vein in C57 BL/6J mice, and repeated every 2 weeks with total 3 doses (top panel). Blood glucose concentrations were measured at the next day following each dose. $n=8$ per group. Data are expressed as group mean $\pm$ SEM. No significant difference is detected in comparison of means among groups of cimagermin alfa administration and vehicle treatment with two-way ANOVA followed by Bonferroni post-test. The overall decline in blood glucose levels over time in all mice may be resulted from a reduction in stress level of mice over the experimental period. This is a typical observation in mice that are initially not adapted to handling, restraint, *iv* injection and blood sampling.

Figure 6. Effects of cimaglermin alfa on blood glucose concentrations in humans with heart failure. Data are expressed as group mean $\pm$ SEM. n=5–6 per group. No statistical difference was detected in comparison of blood glucose levels at indicated time points *vs.* their corresponding pretreatment concentrations by two-way ANOVA followed by Tukey's multiple comparisons test.

Table 1

Insulin and glucagon concentrations (ng/mL) in serum from intracoronary balloon occlusion-induced post-MI pigs treated with cimaglermin alfa at 0.67 mg/kg for a total 8 doses (twice per week, every 3 to 4 days) from 7 to 35 days post-MI (n=7). Samples were collected at baseline and at 50–60 minutes after a single dose of cimaglermin alfa. Data are expressed as group mean $\pm$ SEM. Group means were compared statistically with paired Student *t*-test.

Serum hormone	Concentration pre-infusion (ng/mL)	Concentration post-infusion (ng/mL)	<i>p</i> value
Insulin	0.006 $\pm$ 0.003	0.004 $\pm$ 0.003	> 0.05
Glucagon	1.11 $\pm$ 0.05	1.21 $\pm$ 0.05	> 0.05

Effects of cimagermin alfa on blood glucose concentrations in male and female patients with heart failure. Data are expressed as group mean±SEM if n 3; otherwise, presented with the results from an individual subject (separated by a comma for 2 subjects) or n/a for no subjects. The differences of group means at indicated time points vs. their corresponding pretreatment levels (0 h) were analyzed with repeated one-way ANOVA, and no statistical significance was detected. “n/a” indicates not available.

Table 2

Dose	Gender	n	Blood Glucose (mg/dL) after Dose		
			0 h	0.5 h	2 h
Placebo	Male	4	128±15	124±18	131±17
	Female	2	98, 85	104, 93	85, 93
0.063 mg/kg	Male	4	151±34	147±32	128±20
	Female	1	152	149	103
0.189 mg/kg	Male	2	165, 145	145, 81	118, 119
	Female	3	94±23	68±24	80±9
0.378 mg/kg	Male	5	129±28	121±25	144±32
	Female	0	n/a	n/a	n/a