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Species-specific effects of neuregulin-1β (cimaglermin alfa) on glucose handling in animal models and humans with heart failure

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ABSTRACT

Neuregulin-1β 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-1β, 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-1β 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 h 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 h 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.

1. 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 1β 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 1β have been observed in in vitro studies (Sawyer et al., 2002; Fukazawa et al., 2003). In addition, NRG 1β (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 1β3. 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., 2016), 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,

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cimaglermin alfa and other neuregulins have shown potential as therapeutic agents in stroke (Iaci et al., 2010, 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 1β is involved in the regulation of glucose metabolism (Gumà et al., 2010; Ennequin et al., 2015; Caillaud et al., 2016). NRG 1β 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 1β/ErbB pathway in maintenance of glucose homeostasis (Carver et al., 1997). Thus, NRG 1β 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.

2. Methods

2.1. 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.

2.2. Subjects and procedures

2.2.1. 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 min for a total 8 doses (twice per week, every 3 to 4 days). Blood samples were collected at intervals of 5 or 10 min for the initial 1 h after administration of the last dose for determination of blood glucose levels.

2.2.2. 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 cimaglermin 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 cimaglermin alfa promotes improvement in left ventricular function in rats following MI-induced heart failure (Parry et al., 2016).

2.2.3. 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 and 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 min. Six subjects received placebo, 5 subjects, each, received cimaglermin 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 h after cimaglermin alfa treatment for measurement of blood glucose concentrations.

2.3. 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).

2.4. 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 cimaglermin alfa on glucose disposal. Mice were fasted for approximately 6 h in the morning. Cimaglermin alfa was administered intravenously at doses of either 0.8 or 2.6 mg/kg 4 h 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 h after cimaglermin alfa treatment. Five microliter blood samples were taken immediately before, at 30, 60 and 120 min after the oral glucose challenge to determine blood glucose levels. Areas under the blood glucose concentration-time curve over 120 min were calculated as an index of glucose disposal following vehicle or cimaglermin alfa treatment.

2.5. Insulin and glucagon assay

Blood in pigs treated with cimaglermin alfa at 0.67 mg/kg was sampled before and at 50–60 min 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.