The handle http://hdl.handle.net/1887/30223 holds various files of this Leiden University dissertation

Author: Dongen, Marloes van
Title: Exploring the role of glucagon in glucose homeostasis
Issue Date: 2015-01-07
REFERENCES


ABSTRACT

BACKGROUND Fasting and postprandial hyperglucagonemia in type 2 diabetes mellitus (T2DM) patients cause excessive hepatic glucose production (HGP), suggesting that attenuation of hepatic glucagon action could be a therapeutic strategy for T2DM.

METHODS In this study we evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics in healthy volunteers of single and multiple doses (50 to 400 mg) ISIS 325568, a 2′-O-moe antisense (aso) developed to reduce hepatic glucagon receptor (cccr) mRNA expression. In the multiple dose cohorts, treatment consisted of 8 doses of ISIS 325568 or placebo over 6-weeks. Drug effects were assessed using serial fasting glucagon measurements and the glycemic response to a glucagon challenge at baseline and at the end of 6-week treatment.

RESULTS ISIS 325568 was not associated with clinically relevant changes. Dose-dependent predominantly mild injection site reactions were the most common side-effect. Active treatment caused a gradual increase in fasting glucagon levels and, compared to placebo, a significantly blunt glucagon-induced increase in plasma glucose AUC (24%, p<0.0001) and HGP (13%, p=0.007) at the 400 mg/week dose.

CONCLUSION Six weeks treatment with ISIS 325568 in healthy volunteers attenuated glucagon-stimulated HGP and glucose excursions, supporting further evaluation of the cccr antisense approach in patients with T2DM.

INTRODUCTION

Currently available drug therapy for patients with type 2 diabetes mellitus (T2DM) including the use of insulin is not completely successful in restoring glycemic control. Thus, there remains a need for agents with novel mechanism(s) of action. A possibly feasible approach could be to ameliorate excessive hepatic glucose production. This may be achieved by attenuating glucagon action as it has been shown that in T2DM the glucagon-insulin ratio is disrupted [1] and increased glucagon action is largely responsible for hallmark features of T2DM such as hepatic insulin resistance and increased rates of glucose production [2]. This suggests that targeting glucagon action is a distinct mechanism that may offer therapeutic possibilities (as add-on therapy) for T2DM patients currently uncontrolled with oral anti-diabetic agents. The effect of glucagon are mediated by binding to and activating the glucagon receptor (cccr), which is mainly expressed in the liver and kidney, with lower expression levels in cardiac, adipose and other tissues [3,4]. Pharmacological antagonism of glucagon action has been investigated non-clinically as a potential therapeutic approach for T2DM. Both peptide antagonists and monoclonal antibodies against cccr attenuate hyperglycemia in animal models [5,6]. In addition, antagonism of the cccr in humans suggests that the concept may be worthwhile to pursue [7], but issues relating to pharmacokinetics, selectivity, cross species differences and lack of sustained effects after non-competitive blockade have thus far hindered the development of clinically applicable therapies [8]. An alternative approach could be to use specific antisense oligonucleotides (asos) to the cccr. ISIS 325568 is such an aso targeting cccr mRNA and was designed to function through an rna 1-dependent antisense mechanism [9]. Aso 1 efficiently induces antisense-mediated cleavage of mRNA and is present in most mammalian cells. Antisense-mediated reduction of target mRNA levels by this mechanism results in subsequent reduction in target protein levels [10]. Pharmacology studies with antisense drugs with dedicated (physicochemical) properties have shown that it is possible to achieve tissue-selective inhibition. This was also shown for species-specific cccr antisense drugs including ISIS 325568. It is possible to achieve selective inhibition of hepatic and adipose tissue cccr expression, resulting in normalization of blood glucose levels in diabetic animal models without development of hypoglycemia [11,12]. In addition, cccr antisense therapy increased levels of active GLP-1 levels and improved pancreatic b-cell function [12]. This suggests that specific liver- and fat-directed cccr antisense therapy could offer a novel therapeutic approach in T2DM. Therefore, a study was performed for the first time in human subjects to evaluate the effects of the specific cccr antisense drug ISIS 325568 with the aim to obtain proof of pharmacology in humans while also assessing its tolerability and pharmacokinetics.

METHODS

The study was conducted according to the principles of the Declaration of Helsinki, the Guideline for Good Clinical Practice, and the pertaining Dutch law. The study protocol was approved by the Central Committee on Research involving Human Subjects of the Netherlands. All subjects gave written informed consent before any study-related procedure was performed.

EXPERIMENTAL DRUG – ISIS 325568

ISIS 325568 is the 19 sodium salt of a 3′→5′ phosphorothioate oligonucleotide 20-mer in which each of the 19 internucleotide linkages is an 0-o-linked phosphorothioate diester. There are two 2′-O-(2-methoxyethyl) (moe) modified ribonucleosides at the 3′ and 5′ termini flanking sixteen 2′-deoxyribonucleosides. The sequence of ISIS 325568 is 5′-GMCAMCTTTCTGTCMMe CMeCAGMMeC- 3′ where the underlined bases are 2′-O-moe riboses and the...
cytosine nucleotides are methylated. As such, it is a second-generation antisense phosphorothioate oligonucleotide. In cynomolgus monkeys, which show almost 100% cccr receptor homology at the isis 325568 binding site, isis 325568 was broadly distributed after subcutaneous (sc) injection, mainly to kidney and liver. Full-length isis 325568 is cleared from monkey tissues with a half-life of approximately 11 days in kidney and 13 days in liver. In 13-week mouse and monkey studies, 40 dose-dependently reduced liver cccr mRNA expression to a maximum of 75-90% and resulted in 4- to 6-fold increases over baseline in plasma glucagon and active clp-1 levels. Despite the substantial inhibition of cccr expression, decreases in serum glucose levels below normal were not observed. Apart from the well described common systemic toxicities and side effects (for example, local inflammation resulting in injection site reactions) noted for this generation of asos at doses and exposures that exceed the intended clinical dose level and regimen no adverse findings were noted (13) and data on file. Importantly, no alterations in renal function were observed in monkeys treated with 60 mg/kg/week.

**STUDY POPULATION**

The study was performed in 58 male volunteers who were considered healthy after medical screening. The subjects were between 18 and 65 years of age with a body mass index (BMI) below 30 kg/m², a fasting plasma glucose concentration below 6.4 mmol/L and HbA1c below 6.3%. Main exclusion criteria were clinically significant abnormalities in medical history or physical examination, and abnormalities on laboratory examination.

**PROCEDURES**

The study consisted of two parts. In the first part single sc doses of isis 325568 were administered to 16 volunteers using a double blind design. The doses were 50, 100, 200 and 400 mg with a randomization ratio active:placebo of 3:1. The 50, 100 and 200 mg doses were given as a single sc injection in the abdominal region, and the 400 mg dose was administered as 2 sc injections (200 mg each; one abdominal and one upper arm). Twelve samples for drug assay were taken from the time just prior to injection to approximately 72 hours after injection for pharmacokinetic assessment of the drug. A 24-hour urine sample was also collected from each subject. Also, measurements of vital signs, ecc-parameters, routine laboratory, coagulation and complement factors were performed, and adverse events were recorded. A final follow-up assessment took place at approximately 30 days after dosing.

In the second part of the study 4 sequential cohorts were given 8 doses of study drug over 6 weeks. The doses administered were 50, 100, 200 and 400 mg with a randomization ratio active:placebo of 3:1 for the 50 mg cohort (4 subjects) and 2:1 in the other cohorts (12 subjects in each cohort). In all cohorts, samples for fasting glucagon levels were taken before treatment up to 6 weeks after study drug administration. One week before dosing initiations, subjects in 100, 200 and 400 mg dose cohorts underwent a pre-treatment glucagon challenge. The glucagon challenge was performed as previously described (7;14). The procedure started with a run-in infusion with [6,6-3H] glucose (Cambridge Isotope Laboratories, MA, USA), consisting of a priming dose of 5 mg/kg⁻¹ and a 3 hrs continuous infusion of 0.05 mg/kg⁻¹.min⁻¹. Subsequently, simultaneous infusions of glucagon (3 ng.kg⁻¹.min⁻¹), somatostatin (0.1 µg.kg⁻¹.min⁻¹) and insulin (4 mU.m⁻².min⁻¹) were given for 3 hrs, while the infusion of [6,6-3H] glucose was continued. Samples for (labeled) glucose, insulin and glucagon were taken regularly.

One week later, the subjects were admitted to the clinical center and study drug was given on day 1, 3, and 5 by one hour iv infusions. Thereafter, the subjects received the subsequent 5 sc doses at weekly intervals starting 3 days after the final iv dose. The site of injection alternated between the abdomen and upper arm. One week after the final sc dose a second glucagon challenge was administered. Thereafter, follow-up assessments were done for 9 weeks. Physical examinations, measurement of vital signs, collection of blood specimens for clinical chemistry, hematology, coagulation, and complement tests, collection of urine for urinalysis, ecc measurement, and collection of blood and urine for drug assay were performed regularly throughout the trial. Full plasma pharmacokinetic profiles were obtained following the first intravenous infusion and following the final sc injection. Additional plasma samples were collected to measure trough and peak levels of the subsequent iv infusions. Samples for drug assay were taken just prior to the first, third, fifth and final sc dose. Twenty-four hour urine samples for drug assay were collected following the first and last dose.

**DRUG ASSAY**

Plasma samples were assayed for isis 325568 using a validated hybridization elisa (15). The analysis quantifies the parent compound in plasma with <6% cross-reactivity to oligonucleotide with one base removed (19mer), and no cross-reactivity to the n-2 metabolites of isis 325568 (18mer) and endonuclease shortened oligonucleotides. The calibration range of the assay was 0.5 to 50 ng/mL for isis 325568, with the lower limit defining the lower limit of quantitation.

Urine samples were analyzed after a 2-step solid phase extraction. Analysis was accomplished by capillary gel electrophoresis (cge) with uv detection (260 nm) using a method similar to that previously reported (16). The linear quantification range was from 0.2 to 200 µg/mL, with the lower limit representing the lower limit for quantitation. Metabolite concentrations and total
oligonucleotide concentrations were quantified using calibrators and normalized to the relative extinction coefficients of the putative metabolites and that of the parent compound.

LABORATORY TESTS

Samples for routine clinical chemistry, hematology, and coagulation were analyzed using routine methodology. Insulin was measured with an immunoradiometric assay (Biosource Europe S.A.; interassay coefficient of variation: 6.1-6.5 %), glucagon was measured with a radioimmunoassay kit (LINCO research, Missouri, USA; CV 7.3-13.5 %), and active GLP-1 was measured using the Meso Scale Discovery assay by Pacific Biometrics Inc (Seattle, Washington, USA). The assays were performed in batches to reduce assay variability.

Glucose, labeled [6,6-2H6] glucose concentrations and tracer/tracer ratios were measured using a validated GC/MS assay at the laboratory for endocrinology laboratory of LUMC [17].

For exploratory purposes, IFNG, IL-6, MCP-1, and MIP-1α were measured with ELISA kits that are validated for in vitro diagnostic use, but had low detection limits and reasonable variability. Complement C5a was measured using human C5a ELISA kits (HyCult Biotechnology, Uden, The Netherlands). The placebo and pre-dose data of all subjects in this study showed a mean (SD) C5a concentration of 267 (437) ng/mL. Factor Bb was measured using Quidel Bb Plus Enzyme Immunoassay Kit (Quidel, San Diego, CA, USA). The placebo and pre-dose data of all subjects in this study showed a mean (SD) Bb value of 0.88 (0.22) µg/mL.

STATISTICAL ANALYSIS

The safety and tolerability data analysis was performed on all subjects that were dosed and was descriptive. Pharmacokinetic parameters were obtained from plasma ISIS 325568 concentration-time profiles following single sc injection (first study part), single dose 1-hr iv infusion (first dose second part) and the final sc injection (last dose second part of the study). In addition, urinary excretion of ISIS 325568 and its oligonucleotide metabolites were measured in 0-24 hr collections following both single and multiple dose administration. Plasma pharmacokinetics for ISIS 325568 were analyzed using a non-compartmental method with WinNonLin Professional Version 5.01 software (Pharsight Corp., Mountain View, CA), and included peak plasma concentration (Cmax), time to reach peak plasma concentration (tmax), area under the plasma concentration-time curve extrapolated to infinity (AUC), terminal half-life, and bioavailability (%F). These pharmacokinetic parameters and the urinary excretion values were summarized using descriptive statistics. Dose normalized AUC and Cmax values (normalized to the 200 mg dose) were compared using ANOVA (analysis of variance) to test dose-linearity of exposure.

The analysis on the pharmacodynamic effects of ISIS 325568 was performed on subjects that received all doses and completed the pre- and post-treatment glucagon challenge. The analysis concerned the hepatic glucose production, the systemic glucose disposal, the glucose AUC obtained during the challenges and the difference in fasting glucagon and GLP-1 concentrations during the treatment. Hepatic glucose production was calculated using the Steele equations for non-steady state conditions as adapted for the use of stable isotopes using a pool fraction of 65% [18]. The data was first checked for normality with a Shapiro-Wilk test. Two analyses were performed. The first analysis consisted of a comparison within each dosing group (week 1 versus week 7) with an ANOVA. Results are reported with the estimated differences along with the corresponding 95% confidence interval and p values.

The second analysis consisted of the comparison of active treatment vs. placebo. This was analyzed with a mixed model analysis of variance with treatment, week and time as fixed factors and all the interactions, and subject, subject by week and subject by time as random factors. Results are given as the least squares mean estimates, the estimated difference with the corresponding 95% confidence interval, and the p-value. While it is difficult to draw firm conclusions on the relationship in glucose homeostasis, the glucose AUC during the glucagon challenge was explored. All statistical analyses were done using SAS software (version 9.1.3; Cary, NC).

RESULTS

BASELINE CHARACTERISTICS

Sixteen male subjects were dosed in the single dose part of the study and had an age range of 18 to 63 years and a body mass index (BMI) between 19 and 30 kg/m², and all subjects completed the study. In the multiple dose part of the study a total of 43 male subjects were dosed, ranging in age from 18 to 64 years (mean 39 yrs). The BMI ranged from 18.6 to 29.7 kg/m² (mean 23 kg/m²) and the
baseline glycated hemoglobin levels ranged from 2.7 to 4.2 mmol/mol (mean 33 mmol/mol). During the entire study four subjects were withdrawn; two subjects for non-treatment related intercurrent diseases (anemia, epididymitis), one subject for personal reasons and one subject because of an unsuccessful pre-dose glucagon challenge. The subjects were replaced and the replacements received the same treatment, except for the subject participating in the 50 mg dose group of the multiple dose part of the study who developed anemia.

SAFETY

There were no clinically significant changes in vital signs, ECG recordings and routine laboratory parameters in the single and multiple dose part of the study. In addition, there were no dose-dependent trends or differences in these parameters between placebo-treated and ISIS 325568-treated volunteers. Specifically, renal function parameters such as serum creatinine and urinary excretion of β2-microglobulin (data not shown) were not different between the treatment groups. There was a dose-dependent and transient prolongation of aPTT at 1 hr post iv infusion (maximum average increase of 27 sec in the 400 mg dose group) which had returned to baseline at 3 hrs post infusion. Complement (C3a and Bb), and serum cytokines (IFN-γ, IL-6, MIP-1α, MCP-1) showed that there was no indication for meaningful changes at any dose (data not shown).

The most commonly observed treatment-related AE were predominantly mild injection site reactions (ISRs) that were observed in the multiple dose cohorts, particularly at doses exceeding 100 mg. Injection site reactions occurred as erythema at the sc injection site with or without itch and/or minimal swelling and resolved spontaneously, although in 6 subjects injection site reactions persisted as a small hyperpigmented area. Local lymphadenopathy or other signs of symptoms were not observed.

After iv dosing with 400 mg ISIS 325568, transient feelings of malaise and tiredness, nausea, and headache were observed in 7 of the 8 subjects and was accompanied by fever (max temperature 39.3°C) in 4 subjects. These symptoms were not observed upon any of the sc administrations.

PHARMACOKINETICS

The drug plasma concentration-time curves followed a poly-exponential pattern (Table 1). Distribution half-life was 1-2 hrs following termination of the iv infusion. This rapid distribution phase was followed by at least one much slower elimination phase. Apparent terminal elimination half-lives following the final sc dose administered in the multiple dose cohorts ranged from 14 to 21 days, consistent with slow drug elimination from tissue. Plasma trough levels were stable after the third sc dose suggesting that steady-state levels were reached. Cmax and AUC increased in a dose-dependent and linear fashion over the dose range studied (50 mg to 400 mg), both after the iv and sc single dose administration. Upon repeated dosing, Cmax or AUC did not increase compared to first dose which is consistent with the rapid uptake of the ASo into tissues of distribution and confirms a lack of accumulation in plasma. ISIS 325568 was well absorbed after sc administration with absolute bioavailability ranging between 72-101%. While AUCs were similar following sc injection and iv administration, Cmax following sc administration was approximately 4-fold lower and reached later. Urinary excretion was low, with less than 5% of the administered dose excreted over 24 hr following sc injection. After iv administration of 400 mg approximately 10% of the dose was excreted in urine over 24 hr.

PHARMACODYNAMIC EVALUATION

Fasting plasma glucagon levels increased dose-dependently after multiple doses of ISIS 325568 (Figure 1). The increase in glucagon levels for the integrated response (entire time profile over treatment period) between placebo (60.2 ng/mL) and active treatment was significant for the 400 mg dose (73.9 ng/mL) and amounted to 23% (p=0.017). The baseline corrected average change in fasting plasma glucagon compared to placebo increased for the 400 mg dose only. The change in glucagon concentrations at this dose was 25 ng/mL (p=0.007), while the glucose levels for both treatments were similar (placebo vs. 400 mg treatment: 5.1 vs 5.2 mmol/L before dosing and 5.1 and 4.9 mmol/L after dosing (NS), respectively).

Treatment with CCCa antisense also affected hCG under conditions of selective hyperglycagomina and basal insulin concentrations. Here it was shown that after initiation of the glucagon infusion, plasma glucagon levels rapidly increased and reached a steady-state concentration that was about 2-fold higher than the pre-infusion concentration in all cohorts. During the pre-treatment glucagon challenges, hepatic glucose production (hGP) increased rapidly upon initiation of the glucagon infusion from 1.0 to 2.5 mmol/min (Figure 2). hGP remained 2-fold higher than basal levels for the first 90 minutes of the glucagon infusion and subsequently showed a gradual decline by the end of the glucagon infusion period. The rate of glucose disposal was more or less constant during the glucagon infusion. The glucagon infusion resulted in a rapid elevation of plasma glucose levels from a basal level of 5.5 mmol/L to a steady-state level of 12 mmol/L (Figure 3). This effect was consistent between all the treatment groups. After treatment with ISIS 325568, the glucagon profile was highly similar, but the increase in hGP was blunted, while the rate of disappearance was unaffected (Figure 2 and Table 2). As a consequence, the increase in plasma glucose levels during the hyperglucagomenic period were blunted after the 200 and 400 mg dosing regimen (Figure 3). The effect assessed by the glucose AUC values during the 3-hour challenge was 6% (NS) in the 200 mg group.
5

antisense compounds (data on file). The incidence of injection site reactions appears to be lower with the more recent administration of the same dose. These findings are consistent with previous observations with this class of antisense phosphorothioate oligonucleotide. As there was no decrease in hepatic glucose production. This effect was observed in the 400 mg dose cohort and amounted to approximately 15-20% inhibition in hsp. As in non-human primates ~60% knock-down is associated with a 30% reduction in hsp, it seems that the level of knock-down in humans after treatment with the 400 mg dosage regimen was in the same order of magnitude. Proof of pharmacology in humans was further demonstrated by the gradual increase in fasting glucose levels, a validated biomarker for this target based on preclinical studies. The increase was 23% (ns) in the 200 mg dose group and 41% (p=0.007) for the 400 mg treatment. The most likely explanation of the elevated basal glucagon levels is a feedback of the pancreas as a response to diminished hepatic ccgr receptor availability. However, it can also be a direct effect as pre-clinical studies with cccr asos showed activation of pancreatic alpha cells (evidenced by increased plasma concentrations of active clp-1). As a result of the increase in active clp-1 induced by ccgr aso, islet insulin content is increased, insulin secretion is preserved and glucose tolerance is improved [12]. Importantly, it is unlikely that hypoglycemia resulted in elevated glucagon levels as none of the volunteers had low glucose levels throughout the entire observation period.

Although it is recognized that it is difficult to draw firm conclusions on the pk-pd relationship from this study, it appeared that average trough plasma concentration of isis 325568 correlated with the change in both the pharmacodynamic end-points (plasma glucagon levels as well as reduction in glucose auc during the glucagon challenge). Importantly, plasma trough concentration are reflective of tissue drug concentration and we found that the changes in plasma glucose and glucagon changes in the 400 mg group occurred at average pk trough concentrations of approximately 13 ng/mL and higher. These levels are predictive of liver drug concentrations of 80-100 µg/g liver tissue, which have been shown to produce pharmacology in preclinical models.

In conclusion, 6 weeks treatment with ccgr aso in healthy volunteers resulted in the first demonstration of pharmacology in humans. These results support further evaluation of aso-based therapies against the glucagon receptor in patients with type 2 diabetes mellitus.

DISCUSSION

This study investigated the effects and pharmacokinetics of isis 325568, a second-generation antisense phosphorothioate oligonucleotide. As there was no clinical experience with isis 325568, the dose range chosen for this study which was approximately 0.6 to 6 mg/kg body weight was based on the pre-clinical data of isis 325568, and clinical experience with other 2’-moe-modified asos that were demonstrated to be well-tolerated in healthy volunteers at doses up to 600 mg and treatment durations of 12 weeks [13]. Six weeks of treatment with isis 325568 caused a significant inhibition of the glucagon-mediated increase in hepatic glucose production.

This study extends the safety findings of 2’-moe-modified asos as no clinically relevant changes in vital signs, ecc, clinical chemistry or urinalysis occurred after weekly doses up to 400mg. Particularly, fasting hypoglycemia was not observed and isis 325568 was not associated with untoward renal effects. The latter finding is important as one of the metabolic routes of asos is uptake by proximal tubular cells, followed by sequestration and break-down in lysosomes. Apparently, accumulation of isis 325568 in the proximal tubules in humans does not affect renal function, which is consistent with the findings in animals. The flu-like symptoms, which possibly reflect a mild systemic inflammatory reaction, were observed at the 400 mg iv dose only and not after sc administration of the same dose. These findings are consistent with previous observations with this class of asos [13;19]. Local injection site reactions occurred frequently at weekly doses ≥ 100 mg with an incidence of ~60%. The incidence of injection site reactions appears to be lower with the more recent antisense compounds (data on file isis Pharmaceuticals Inc., usa).

The pharmacokinetics of isis 325568 showed an almost complete bioavailability after sc dosing with terminal elimination half-lives in order of 14-21 days, consistent with slow drug elimination from tissue. Cmax and auc increased in a dose-dependent and linear fashion over the dose range studied (50 mg to 400 mg), both after the iv and sc single dose administration. However, consistent with the rapid uptake of the aso into tissues, Cmax or auc did not increase upon repeated dosing. Urinary excretion over 24 hr amounted to only ~5% of the administered dose, with a trend for higher excretion (~10%) after iv administration of the 400 mg dose. The latter observation suggests a higher unbound fraction in plasma available for filtration.

This study showed that 6 weeks of treatment with isis 325568 translated into a significant inhibition of the glucagon-mediated increase in hepatic glucose production. This effect was observed in the 400 mg dose cohort and amounted to approximately 15-20% inhibition in hsp. As in non-human primates ~60% knock-down is associated with a 30% reduction in hsp, it seems that the level of knock-down in humans after treatment with the 400 mg dosage regimen was in the same order of magnitude. Proof of pharmacology in humans was further demonstrated by the gradual increase in fasting glucose levels, a validated biomarker for this target based on preclinical studies. The increase was 23% (ns) in the 200 mg dose group and 41% (p=0.007) for the 400 mg treatment. The most likely explanation of the elevated basal glucagon levels is a feedback of the pancreas as a response to diminished hepatic ccgr receptor availability. However, it can also be a direct effect as pre-clinical studies with ccgr asos showed activation of pancreatic alpha cells (evidenced by increased plasma concentrations of active clp-1). As a result of the increase in active clp-1 induced by ccgr aso, islet insulin content is increased, insulin secretion is preserved and glucose tolerance is improved [12]. Importantly, it is unlikely that hypoglycemia resulted in elevated glucagon levels as none of the volunteers had low glucose levels throughout the entire observation period.

Although it is recognized that it is difficult to draw firm conclusions on the PK-PD relationship from this study, it appeared that average trough plasma concentration of isis 325568 correlated with the change in both the pharmacodynamic end-points (plasma glucagon levels as well as reduction in glucose auc during the glucagon challenge). Importantly, plasma trough concentration are reflective of tissue drug concentration and we found that the changes in plasma glucose and glucagon changes in the 400 mg group occurred at average pk trough concentrations of approximately 13 ng/mL and higher. These levels are predictive of liver drug concentrations of 80-100 µg/g liver tissue, which have been shown to produce pharmacology in preclinical models.

In conclusion, 6 weeks treatment with ccgr aso in healthy volunteers resulted in the first demonstration of pharmacology in humans. These results support further evaluation of aso-based therapies against the glucagon receptor in patients with type 2 diabetes mellitus.

EXPLORING THE ROLE OF GLUCAGON IN GLUCOSE HOMEOSTASIS

FIRST INTO MAN OF A GLUCAGON RECEPTOR ANTAGONIST DRUG
### Table 1
Mean ± standard deviation of pharmacokinetic parameters for isis 325568 after the initial 1-hour intravenous infusion (A) and final sc administration (B) in the multiple dose part of the study.

<table>
<thead>
<tr>
<th></th>
<th>50mg (n=3)</th>
<th>100mg (n=10)</th>
<th>200mg (n=8)</th>
<th>400mg (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>8.2 ± 0.6</td>
<td>14 ± 1.6</td>
<td>30 ± 7.0</td>
<td>54 ± 18</td>
</tr>
<tr>
<td>AUC0-inf (µg*hr/mL)</td>
<td>16 ± 2.0</td>
<td>33 ± 4.5</td>
<td>66 ± 9.3</td>
<td>121 ± 14</td>
</tr>
<tr>
<td>Cl (L/hr)</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Vss (L)</td>
<td>6.5 ± 0.9</td>
<td>6.7 ± 1.0</td>
<td>7.5 ± 1.0</td>
<td>9.1 ± 1.2</td>
</tr>
<tr>
<td>T1/2, distribution (hr)</td>
<td>2.0 ± 0.04</td>
<td>2.2 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>T1/2, final (hr)</td>
<td>8.2 ± 0.2</td>
<td>7.8 ± 1.2</td>
<td>6.4 ± 0.7</td>
<td>6.4 ± 2.1</td>
</tr>
</tbody>
</table>

**A (IV)**

<table>
<thead>
<tr>
<th></th>
<th>50mg (n=2)</th>
<th>100mg (n=8)</th>
<th>200 mg (n=8)</th>
<th>400 mg (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>1.9</td>
<td>3.1 ± 0.9</td>
<td>7.0 ± 1.6</td>
<td>9.2 ± 2.2</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>4.0</td>
<td>3.4 ± 2.0</td>
<td>2.6 ± 1.0</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>AUC0-24h (µg*hr/mL)</td>
<td>15</td>
<td>23 ± 3.9</td>
<td>51 ± 6.6</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>AUC0-168h (µg*hr/mL)</td>
<td>16</td>
<td>25 ± 4.3</td>
<td>56 ± 6.5</td>
<td>88 ± 19</td>
</tr>
<tr>
<td>% F</td>
<td>101</td>
<td>76 ± 11</td>
<td>86 ± 13</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>T1/2, distribution (hr)</td>
<td>2.3</td>
<td>3.3 ± 1.1</td>
<td>3.4 ± 1.1</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>T1/2, final (days)</td>
<td>14</td>
<td>21 ± 8.9</td>
<td>19 ± 3.4</td>
<td>18 ± 11</td>
</tr>
</tbody>
</table>

**B (SC)**

### Table 2
Mean (sd) values in hepatic glucose production (hgp), glucose rate of disappearance (Rd), and time-corrected area under the curve for glucose (AUC/T) during the hyperglucagonemic challenge before (pre) and after (post) treatment for 8 weeks with different dose of isis 325568 or placebo. The point estimate of the difference along with the corresponding 95% confidence interval (95% CI) and p-value are reported.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=12)</th>
<th>100mg isis 325568 (n=8)</th>
<th>200mg isis 325568 (n=8)</th>
<th>400mg isis 325568 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGP (µmol/min)</td>
<td>1525 ± 246.0</td>
<td>-35 (-244 / 174)</td>
<td>-96 (-296 / 103)</td>
<td>-282 (-482 / -82)</td>
</tr>
<tr>
<td>Rd (µmol/min)</td>
<td>1318 ± 166.1</td>
<td>-14 (-298 / 269)</td>
<td>-21 (-196 / 238)</td>
<td>-32 (-227 / 213)</td>
</tr>
<tr>
<td>AUC/T (mmol/L)</td>
<td>9.6 ± 1.2</td>
<td>0.1 (-0.9 / 1.1)</td>
<td>0.1 (0.2 / 1.0)</td>
<td>0.1 (-0.1 / 0.1)</td>
</tr>
</tbody>
</table>

Cmax: maximal concentration; AUC0-24h/0-168h: Area Under the Curve for t= 0 min to infinity, 24 hrs, 168 hrs; Cl: Clearance; Vss: volume of distribution; T ½, distribution/final: half-life associated with distribution/final phase; % F: percentage bioavailability.
**FIGURE 1** Time course of plasma glucagon levels (mean and so) during multiple dose treatment with ISIS 325568. Intravenous doses are indicated by the dashed arrows and solid arrows indicate the weekly sc doses. For clarity the so bars are given for the placebo and 400 mg dose regimen only.

**FIGURE 2** Time course of hepatic glucose production (HGP) and rate of glucose disposal (Rd) before and after 8 weeks treatment with placebo (left panel; n=13) or 400 mg ISIS 325568 (right panel; n=8).

**FIGURE 3** Mean glucose values during glucagon challenge for the different dose groups before (closed circles) and after 8 weeks treatment (open squares) with ISIS 325568.
FIGURE 4 Plots of steady state trough concentration of ISIS 325568 after 6 weeks of treatment versus the change in glucose AUC during the glucagon challenge (A) and change in glucagon (B).

REFERENCES
CHAPTER 6

Modeling the effect of a glucagon challenge on glucose homeostasis in humans

M.G.J. van Dongen¹, R. Alvarez-Jimenez¹, J. Stevens¹, L.A. Peletier², A.F. Cohen¹, J. Burggraaf¹

1. Centre for Human Drug Research (chdr), Leiden, The Netherlands
2. Mathematical Institute, Leiden University, Leiden, The Netherlands