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ORIGINAL ARTICLE

Novel SGLT2 inhibitor: first-in-man studies of antisense compound is associated with unexpected renal effects

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Abstract

The antisense compound ISIS 388626 selectively inhibits renal glucose reabsorption by inhibiting the sodium–glucose cotransporter-2 (SGLT2) mRNA expression. It is developed as an insulin-independent treatment approach for type 2 diabetes mellitus (T2DM). The safety, tolerability, pharmacokinetics, and pharmacodynamics after subcutaneous administration of the drug were planned to be evaluated in healthy volunteers in a single-ascending-dose study (50–400 mg) and a multiple-ascending-dose study (6 weeks; weekly doses of 50–400 mg with loading dose regimen of three doses during the first week). The study was halted early because increases in serum creatinine occurred in the subjects participating in the 100 mg multiple-dose cohort. The pronounced changes in serum creatinine were accompanied by increased urinary excretion of beta-2-microglobulin and KIM1. The possible mechanisms for these findings remain elusive and are in contrast to preclinical findings as comparable treatment with ISIS 388626 of animals did not reveal similar changes. Although exposure was limited, there was an indication that glucosuria increased upon active treatment. Before the concept of antisense-mediated blocking of SGLT2 with ISIS 388626 can be explored further, more preclinical data are needed to justify further investigations.

Abbreviations

AE, Adverse events; aGST, alpha-glutathione S-transferase; LCRIS, local cutaneous reactions at the injection site; MAD, multiple ascending doses; NAG, N-acetyl-β-(D)-glucosaminidase; NIDDM, non-insulin-dependent diabetes; OGTT, oral glucose tolerance test; SAD, single ascending doses; UGE, urinary glucose excretion.

Introduction

The renal SGLT2 transporter accounts for 90% of the reabsorption of glomerular filtrated glucose (Hummel et al. 2011). In non-insulin-dependent diabetes (NIDDM), insulin resistance and the subsequent chronic hyperglycemia increases the amount of filtrated glucose. NIDDM is associated with increased SGLT2 transporter expression and activity, thereby contributing to the hyperglycemic state (Rahmoune et al. 2005). These findings render the SGLT2 transporter a promising target for patients with diabetes mellitus type 2. Indeed, inhibition of SGLT2 using small molecules has proven its efficacy by improving glycemic control in subjects with type 2 diabetes (Bailey et al. 2012; Riser and Harris 2013). In addition, this treatment improves other cardiovascular risk measures such as blood pressure, weight, and levels of triglycerides (Riser and Harris 2013). This has led to the registration of several drugs dapagliflozin (Plosker 2012) (FORXIGA®), canagliflozin (Sha et al. 2014) (Invokana®), and empagliflozin (Jahagirdar and Barnett 2014) (Jardiance®). It was recently shown that adding empagliflozin to standard care favors cardiovascular outcome (Zinman et al. 2015). The use of clinically relevant doses of these compounds result in a modest 30–50% inhibition of renal glucose reabsorption (List et al. 2009; Liu et al. 2012). An alternative approach is reduction in the synthesis of the transporter by antisense interference

which theoretically could reduce the transporter to 80–90%. Targeting the kidney with antisense oligonucleotides should be possible as these compounds generally distribute to the kidney (Oberbauer et al. 1995). One of the antisense compounds developed to selectively knockdown the SGLT2 receptor is ISIS 388626, a second-generation 2′-methoxyethyl (MOE)-modified 12-mer phosphorothioate oligonucleotide. This oligonucleotide is structurally complementary to a portion of the coding region of SGLT2 mRNA in multiple species including, mouse, rat, rabbit, monkey, dog, and human. This compound was developed to inhibit the synthesis of the renal SGLT2 receptor by utilizing its short length (12-mer vs. the typical 20-mer), that enables fractional glomerular clearance and thus selective targeting of the proximal tubular epithelium (Zanardi et al. 2012).

Weekly subcutaneous (sc) injection of ISIS 388626 showed to be an effective and safe treatment in preclinical studies ranging from 6 weeks to 6 months in duration with a dose range of 0–30 mg/kg/week (Zanardi et al. 2012; Bhanot 2009). Following sc injection in animals, peak plasma concentrations and AUC increased in a dose-dependently and were similar for single and repeated dosing for up to 13 weeks in mice and monkeys. Peak plasma concentrations occurred within 0.25–1.5 h after sc injection in mice, rats, and monkeys, then decreased in an apparent multi-exponential fashion with time. ISIS 388626 was initially predominantly cleared by distribution to the kidneys with a plasma half-life (t1/2α) ranging between 1.6 and 2.9 h. The initial rapid distribution phase was followed by a slow elimination phase with an elimination half-life between 5 and 8 days. In dogs, diabetic rodents, and monkeys, ≥80% reduction of renal SGLT2 mRNA expression was observed without affecting SGLT1 expression (Bhanot 2009). In normoglycemic animals, the reduction in SGLT2 mRNA expression that occurred at doses of 1–3 mg/kg weekly for 13 weeks translated into effective glucosuria (at 3 mg/kg a 60-fold increase in mice and 7-fold increase in monkeys in urine glucose creatinine ratio) (Zanardi et al. 2012). Interestingly, ISIS 388626 also slowed progression of ocular cataract formation, glomerular damage, and pancreatic islet cell deterioration in diabetic rodents (Wancewicz 2008). Signs of toxicity of ISIS 388626 were not observed in any of the animal models. In a 6-month study in diabetic rats, no accumulation of ISIS 388626 was observed in cardiac, liver, or intestinal tissues, demonstrating the specificity and selective renal distribution of ISIS 388626 (Wancewicz 2008). In 6 to 13 weeks treatment studies in mice and monkeys, the only histological changes consisted of dose- dependent accumulation of basophilic granules in tubular epithelial cells, which is a result of H&E staining of the oligonucleotide itself in the cytoplasm and is an expected effect (Monteith and Levin 1999; Rappaport et al. 1995). Also, no indications for long-term changes of general kidney function were noted (Zanardi et al. 2012).

Based on this preclinical information, it was considered safe to evaluate the compound in humans. Here, we describe the results of the first in human trial with ISIS 388626, performed to assess its effects after single ascending doses (SAD) and multiple ascending doses (MAD).

Materials and Methods

Subject

Inclusion and exclusion criteria were similar for both parts of the trial. Adult, male or (postmenopausal or surgically sterile) female subjects (18–65 years) with BMI < 30 kg/m^2 were eligible if fasting plasma glucose and HbA1C was below the upper limit of normal and it was agreed to maintain steady hydration throughout study participation. Excluded were pregnant or nursing women, and subjects with significant abnormalities regarding medical history, physical examination findings, 12-lead electrocardiogram findings, and clinical laboratory evaluations (including positive protein in urine dipstick analysis and calculated eGFR below 60 mL/min (Levey et al. 1999). Studies were conducted in accordance with good clinical practice guidelines and were approved by the national ethics committee as well as the competent authority.

Choice of dosing

It was anticipated to explore doses of 50, 100, 200, and 400 mg of ISIS 388626. The doses were based on a MABEL approach, taking into account a No Adverse Effect Level estimated to be 10 mg/kg/week (including a loading dose regimen) in monkeys. In preclinical studies across multiple species, the pharmacologically active dose range of ISIS 388626 was 1–3 mg/kg/week. At this exposure, a significant reduction in SGLT2 mRNA occurred (74–97% in mice and approximately 30 to 90% in monkeys over the dose range 1–30 mg/kg/week), accompanied by a 25–200 fold increase in urinary glucose excretion (Zanardi et al. 2012; Bhanot 2009; Wancewicz 2008). Based on this, estimation of the equivalent human effective dose falls in the range of 1–3 mg/kg/week. Experience with other 2′-MOE-modified antisense oligonucleotides, safely administered (intravenously and subcutaneously) in multiple clinical studies at doses up to weekly 750 mg (which translates into 10.7 mg/kg/week assuming an average weight of 70 kg), with treatment durations exceeding 1 year (Kwoh and Crooke 2007), further supports the safety of this dose range. In the MAD part of the study, it was planned to

administer these doses weekly for 6 weeks after a loading dose regimen of three doses in the first week to achieve effective steady-state tissue concentrations.

Study design

The first study was a double-blind, randomized, placebo-controlled single ascending dose (SAD) study. Sixteen subjects were randomly assigned in a 3:1 ratio to receive either a single dose of 50, 100, 200, or 400 mg ISIS 388626 or placebo, administered as sc injection in the abdominal region. The SAD study part was followed by a double-blind, randomized, placebo-controlled multiple ascending dose (MAD) study administered as subcutaneous injection in the four abdominal quadrants (i.e., upper left, upper right, lower right, lower left), or upper lateral arms and thighs. Subjects were to receive eight doses of study drug (or placebo) over a 6 week period, three doses in study week 1 followed by once weekly dosing for 5 weeks. At the higher dose levels in this study part (>50 mg), the intended pharmacodynamic effects of ISIS 388626 (inhibition of renal urinary glucose reabsorption and lowering of plasma glucose concentrations) were to be estimated by evaluation of glucose handling after an oral glucose tolerance test (OGTT), performed before the first administration of ISIS 388626/placebo and at Week 6. In both study parts, dose escalation was only permitted when the preceding dose regimen did not raise any safety concerns.

Safety measurements

The safety assessments were similar for both study parts and consisted of recording adverse events and measurement of vital signs, electrocardiograms, physical examinations, and clinical laboratory tests (including clinical chemistry, hematology, coagulation, complement tests, and urinalysis (including excretion of B2M)) throughout the study period. Adverse events were captured in MedDRA terms.

Renal markers

The biomarkers beta-2-microglobulin (B2M), kidney injury molecule (KIM1), alpha-glutathione s-transferase (aGST), and N-acetyl-β-(d)-glucosaminidase (NAG) were chosen based on their performance on detecting injury to the proximal tubule where SGLT2 is located (van Meer et al. 2014). Analysis of renal damage markers aGST and NAG was performed using quantitative enzyme immunoassays (NEPHKITO immunoassay for aGST, Argutus Medical Dublin, Ireland, and Diazyme 70010 Rev. F (Poway, USA) for NAG). KIM1 was measured using a microsphere-based immunoassay.

Pharmacokinetic analysis

For the quantification of ISIS 388626, plasma samples were collected frequently after administration of the single dose for 48 h after first and sixth dose in the multiple dose part. In the MAD part, samples were also taken before dosing in week 2 and 4 and during 5 weekly follow-up visits). In addition, PK urine collections were done after first and sixth dose (up to 24 and 48 h post-dose). Plasma samples were analyzed using a validated hybridization enzyme-linked immunosorbent assay and urine samples were analyzed by a validated capillary gel electrophoresis method. Both assays were performed at PDD laboratories Middleton, WI (Richmond, USA).

The plasma pharmacokinetics of ISIS 388626 were evaluated using noncompartmental analyses. The analyses were performed to determine the maximum observed plasma concentration (C_{max}), the time to maximum plasma concentration (T_{max}), and the area under the plasma concentration-time curve from dosing to 48 h after dosing ($AUC_{0\text{-}48h}$) using WinNonLin (version 5.3, Pharsight Corporation, Mountain View, CA, USA).

Data analyses and statistical methods

Safety and tolerability evaluation was based on descriptive statistics

The sample sizes (four subjects per cohort for the SAD part, eight subjects per cohort for the lowest dose range in the MAD part) were selected to allow descriptive analysis of safety and pharmacodynamics of ISIS 388626, and were not supported by any statistical rationale. The sample size of 12 subjects for the cohorts treated at dose levels exceeding 50 mg ISIS 388626 was based on observed changes in plasma glucose ($AUC_{0\text{-}120min}$) upon an OGTT challenge in healthy volunteers, with an estimated standard deviation 85 mmol*min/L. At a sample size of six subjects per treatment group, at least 80% power would be achieved to detect a 170 mmol*min/L difference in plasma glucose $AUC_{0\text{-}120min}$ between treatment groups at an alpha level of 0.05. Based on this power calculation, a sample size of 12 was selected to ensure sufficient power.

The pharmacodynamic evaluation was based on descriptive summary statistics only for the MAD 50 mg cohort, as previous experience with second-generation oligonucleotides suggests that the minimal pharmacologically effective dose exceeds 100 mg/week (Kastelein et al. 2006).

Pharmacodynamic effects of ISIS 388626 at dose levels above 50 mg were statistically evaluated using a two-sided *T*-test, with placebo subjects from different cohorts

pooled as a group. Serum glucose, 24 h urinary glucose excretion (UGE), and fractional glucose excretion (defined as (UGE/ filtered glucose load (GFR*fasted plasma glucose) *100) at the end of treatment (week 6) were compared between ISIS 388626 treatment groups and placebo group. For the cohorts with doses of 100, 200, and 400 mg, it was planned to analyze change from baseline of the pharmacodynamic endpoints to Week 6 assessments among treatment groups using ANOVA.

Results

Subject

The study was performed at the Centre for Human Drug Research in the Netherlands. Sixteen subjects were enrolled and completed the SAD study. Twenty-three subjects enrolled the MAD study, of whom all 8 subjects assigned to the 50 mg cohort completed the study (6 active 2 placebo) and 15 subjects assigned to the 100 mg cohort terminated early due to a premature halt of the study (12 active, 3 placebo). The six actively treated subjects of the 50 mg cohort received all eight doses. In the 100 mg cohort, the first four doses were received by all 12 actively treated subjects. Thereafter, eight subjects received the fifth dose and four subjects received the sixth dose and no subjects in the 100 mg cohort received the seventh or eighth dose. Subject demographics are presented in Table 1.

Table 1. Summary of subject demographics.

	Number	Age (years ± SD)	BMI (Kg/m^2 ± SD)	% Male
SAD 50 mg	3	31.3 ± 13.7	23.6 ± 1.7	100
SAD 100 mg	3	22.0 ± 5.3	23.4 ± 2.1	100
SAD 200 mg	3	24.7 ± 5.0	23.5 ± 1.5	100
SAD 400 mg	3	26.7 ± 8.0	24.3 ± 1.5	100
SAD PLACEBO	4	21.0 ± 0.8	21.3 ± 0.8	100
MAD 50 mg	6	50.7 ± 16.4	24.6 ± 3.0	83.3
MAD 100 mg	12	40.1 ± 14.4	23.9 ± 2.8	83.3
MAD PLACEBO	5	40.8 ± 16.4	25.4 ± 3.3	100

SAD, single ascending dose; MAD, multiple ascending dose.

Safety outcomes

Adverse events (AEs) were reported in 26 (87%) subjects who received single and multiple doses of ISIS 388626, and in 6 (67%) subjects who received placebo. All AEs reported were classified as mild in intensity and were transient. The most common AE was fatigue which was reported by 18 of 30 subjects (60%) treated with single or multiple sc doses of ISIS 388626. Incidence of this AE did not increase as dose increased. Fatigue was also reported in the placebo group in two of nine subjects (22%). Other than fatigue, the only other AEs that were reported more than once were headache (13%), nasopharyngitis (33%), and dizziness (10%). These adverse events are unlikely to be drug-related as these AEs occurred in placebo and both dose groups with a similar incidence (Table 2). No hypoglycemia occurred at any dose level. In the MAD part of the study, injection site reactions (ISRs) were observed in the treated groups. When expressed as local cutaneous reactions at the injection site (LCRIS; defined as erythema, swelling, itching, pruritus, pain or tenderness with an onset on the day of injection which did not resolve on the day of the injection or the day after the injection), the above mentioned skin reactions occurred in 2 out of 6 subjects at doses of 50 mg and in 6 out of 12 subjects at doses of 100 mg ISIS 388626. All ISRs were mild in severity. Most ISRs resolved completely and spontaneously during the study period with a duration ranging from 14 days–2 months. In the two female subjects, the ISRs did not completely resolve before the last follow-up visit. The ISRs were not progressive, not accompanied by local lymphadenopathy, and no study discontinuations occurred due to ISRs.

In the SAD study, no clinically relevant changes in laboratory findings, vital signs, ECG-derived parameters, or body temperature were observed. However, marked changes were observed in serum creatinine levels for several subjects of both cohorts of the MAD study. In the 50 mg cohort, a minimal increase in average serum creatinine of 0.13 ± 0.09 mg/dL (13% increase) was observed during the first 3 weeks after treatment initiation (Fig. 1), with increases over baseline in individuals ranging from

Table 2. Frequency overview of adverse events reported more than once (%).

	SAD 50–400 mg (n = 12)	SAD PLACEBO (n = 4)	MAD 50 mg (n = 6)	MAD 100 mg (N = 12)	MAD PLACEBO (N = 5)
Fatigue	50	25	66.7	66.7	20
Headache	0	0	33.3	16.7	40
Nasopharyngitis	8.3	0	0	33.3	60
Dizziness	0	0	0	25	40
ISR's	0	0	33.3	50	0

SAD: single ascending dose. MAD: multiple ascending dose.

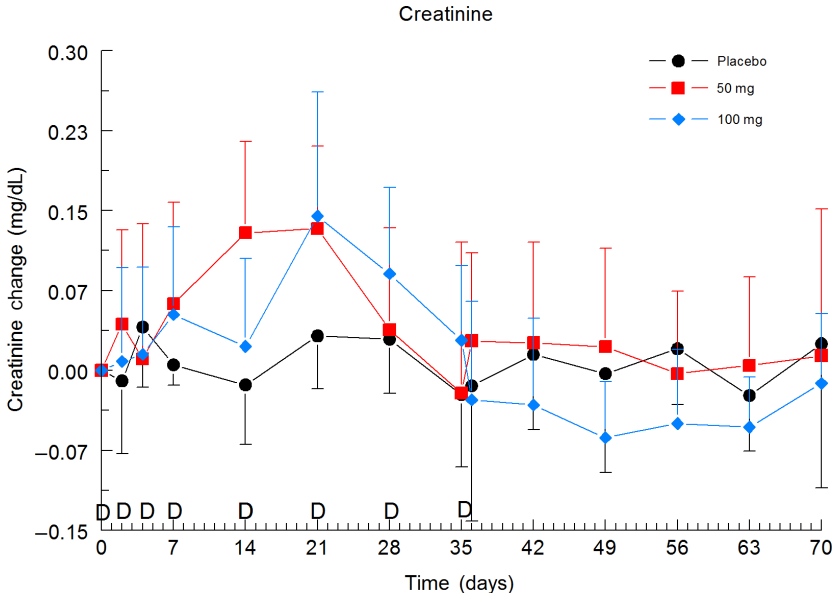

Figure 1. Average change from baseline of serum creatinine over time in the multiple ascending dose study with SD error bars. Timing of dosing is indicated with D's. The six subjects of the 50 mg cohort received all eight doses. In the 100 mg cohort, the first 4 doses were received by all 12 subjects. Thereafter, nine subjects received the fifth dose and four subjects received the sixth dose and no subjects in the 100 mg cohort received the seventh or eighth dose, due to the early halt of the study. In the 50 mg cohort, a minimal increase in average serum creatinine of 0.13 ± 0.09 mg/dL (13% increase) was observed during the first 3 weeks after treatment initiation. The values returned to baseline levels after the third treatment week, despite continued dosing. In the 100 mg cohort, an increase in average serum creatinine of 0.14 ± 0.12 mg/dL (16% increase) was observed during the first 3 weeks.

0.11 to 0.23 mg/dL. Since the individual changes in serum creatinine values were not considered clinically significant, study drug administration in this cohort was continued. Indeed, serum creatinine values returned to baseline levels after the third treatment week, despite continued dosing (Fig. 1). Subjects did not have any other clinically significant finding until follow-up, including no change in serum electrolytes or proteinuria. Variability in serum creatinine over time was large, as demonstrated by the placebo-treated group, with changes over baseline in serum creatinine levels ranging from −0.11 to 0.21 mg/dL. Nonetheless, the changes in serum creatinine observed in the ISIS 388626 treated subjects were more pronounced compared to the placebo group. In the 100 mg cohort, during the first 3 weeks of study drug treatment, an increase in average serum creatinine of 0.14 ± 0.12 mg/dL (16% increase) was observed compared to baseline values, ranging on an individual level from 0 to 0.47 mg/dl (Fig. 1). As the interindividual variability in serum creatinine upon ISIS 388626 treatment was high, supplemental individual narratives are provided below, focusing on two subjects with the most pronounced increase in serum creatinine level.

The changes in serum creatinine were often accompanied by a small rise in BUN without clinically meaningful changes in serum electrolytes, albumin, aldosterone, or plasma renin activity. Besides creatinine and BUN, no other chemistry parameter, including parameters of liver biochemistry changed after dosing. Urine flow and urinalysis parameters did not change significantly in the subjects with increased creatinine, except for one subject, whose urinary protein excretion was 1.48 g/24 h after the fourth dose (Week 2) (See narratives below).

Based on the observed creatinine increases in the running 100 mg cohort, further dosing of all subjects was discontinued, for safety reasons. All 15 subjects of the 100 mg cohort (12 on active treatment and 3 on placebo) entered the follow-up period. Therefore, it was impossible to assess if continued dosing in the 100 mg dose group would have resulted in a resolution of the renal effect as observed in the 50 mg cohort.

To provide mechanistic insight into the observed changes in serum creatinine, analysis of potential renal damage markers KIM1, NAG, alpha-GST (aGST), and beta-2-microglobulin (B2M) was performed in biobanked urine samples (Fig. 2). After four doses, a dose-dependent increase in B2M excretion was observed in nearly all ISIS 388626-treated subjects. In the 50 mg cohort, an average of 843 ± 1027.5 μg/24 h was observed, versus 69.8 ± 27.6 μg/24 h in the placebo group. For the 100 mg cohort, the increase was even more pronounced with an average of 2200 ± 2956.2 μg/24 h (versus

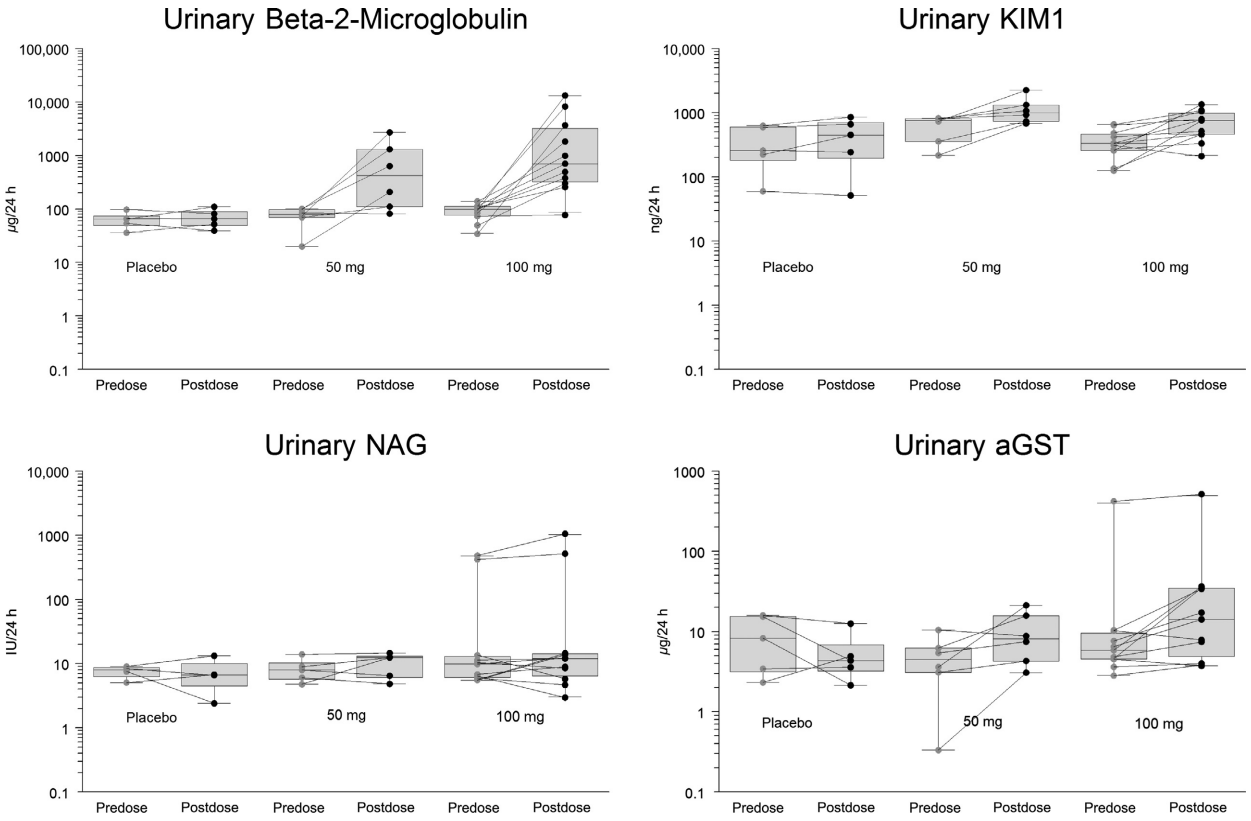

Figure 2. Box-Whisker plot of urinary renal damage markers Pre-Dose and Post-Dose (after 4 doses). The bottom and top of the box are the 25th and 75th percentile, respectively and the band inside is the median. The ends of the whiskers represent the minimum and maximum of all the data. Dots are individual results, grey being pre-dose measurements, black being values measured after 4 doses of study drug. After 4 doses, a dose-dependent increase in B2M excretion was observed in nearly all ISIS 388626-treated subjects. An average of 843 ± 1027.5 ug/24 hrs was observed for the 50 mg cohort and 2200 ± 2956.2 ug/24 hrs for the 100 mg cohort, versus 69.8 ± 27.6 ug/24 hrs in the placebo group. Also KIM 1 and aGST excretion increased upon ISIS 388626 treatment in the majority of treated subjects, although there were no clear dose relationships observed for these renal markers. No clear changes occurred in urinary NAG.

69.8 ± 27.6 μg/24 h in the placebo group). Also, KIM1 and aGST excretion increased upon ISIS 388626 treatment in the majority of treated subjects, although there were no clear dose relationships observed for these renal markers. No significant changes were observed in urinary NAG excretion upon ISIS 388626 treatment. B2M, KIM1, and aGST excretion returned to baseline levels during follow-up (data not shown). No significant changes in absolute creatinine clearance were observed, however inter- and intrasubject variability was large (data not shown).

Individual subject narratives

A 63-year-old female received four doses of ISIS 388626 at a dose level of 100 mg (Day 1, 3, 5 and 8). A 75% increase in serum creatinine level was observed in a blood sample collected prior to administration of the fourth dose (1.1 mg/dL versus 0.61 mg/dL at baseline). There were no additional clinically remarkable findings, but monitoring of the subject was intensified. Three days after administration of the fourth dose, total urine protein excretion was 1.5 g/24 h, and further treatment was discontinued. During the intensive follow-up period, serum creatinine levels and urine protein excretion decreased and returned to baseline levels within 6 weeks after the last drug administration. A week after the last drug administration, glucosuria (approximately 1200 mg/24 h) was observed. Post hoc analysis showed that creatinine changes were accompanied by transient increases of potential renal damage markers (B2M, NAG, and KIM1), that also returned to baseline during the follow-up period. Aside from the transient serum creatinine and urine protein elevations, other adverse events for this subject (obstipation, fatigue, common cold, and ISRs at the four injection sites) were of mild intensity and resolved without intervention. The subject's ISIS 388626 AUC_{0-48h} levels after the first dose and trough levels on Day 3, 5, and 8 were comparable to the other subjects in the 100 mg treatment group.

In a 39–year-old male who received five doses of ISIS 388626 at a dose level of 100 mg (day 1, 3, 5, 8, and 15), a 54% increase in serum creatinine level was observed prior to administration of the fifth dose (1.39 mg/dL vs. 0.90 mg/dL at baseline). ISIS 388626 treatment was discontinued and an intensive follow-up period was started in which serum creatinine values returned to baseline levels. Renal damage markers (proteinuria, B2M and aGST) transiently increased, peaking after the fourth dose and returning to baseline levels during the follow-up period. No change in urinary glucose excretion was observed in this subject. Aside from the serum creatinine elevations, the other adverse events (one ISR and migraine-like symptoms after the first dose) relieved with paracetamol and ibuprofen treatment) were of mild intensity. Also for this subject, ISIS 388626 AUC_{0-24h} after the first dose and trough levels on day 3, 5, and 8 were comparable to the other subjects in the 100 mg treatment group.

Pharmacokinetics

Following single sc injection, ISIS 388626 was rapidly absorbed as demonstrated by reaching maximum plasma concentrations (C_{max}) between 1.2 and 1.5 h (Fig. 3, Table 3). Plasma concentrations of ISIS 388626 decreased rapidly after reaching C_{max}, and distributed to tissues. The total amount of ISIS 388626 in urine increased more than dose-proportionally (Fig. 4).

After single administration of ISIS 388626, AUC increased in a dose-proportional manner, whereas the increase in C_{max} was less than dose-proportional (Table 3). Repeated pharmacokinetics in the multiple dose study part could only be assessed for the 50 mg dose, as other dose levels were prematurely terminated or not executed. The PK analysis showed that no accumulation of ISIS 388626 occurred with repeated dosing, as demonstrated by Cmax and AUC (Table 4). Plasma pharmacokinetic parameters were comparable between the first dose (day 1) and last dose (day 36). The differences in plasma AUC and Cmax between subjects in the single-dose cohort and multiple-dose cohorts are likely explained by intersubject variability.

Pharmacodynamics

Due to the early halt of this part of study, the statistical analysis was constrained and a formal efficacy analysis could not be performed. Descriptive statistics are provided, describing effects of ISIS 388626 on UGE, FI and serum glucose following the OGTT in the MAD 50 mg cohort, and the MAD 100 mg cohort that was halted prematurely. Repeated administration of 50 mg ISIS 388626

Table 3. Plasma pharmacokinetics single ascending dose study. *N*=number

Dose	*N*	AUC 0–48 h (μg*h/mL)	C_{max} (μg/mL)	T_{max} (h)
50 mg	3	5.82 ± 2.18	1.31 ± 0.59	1.17 ± 0.58
100 mg	3	13.0 ± 0.78	1.72 ± 0.35	1.17 ± 0.76
200 mg	3	22.2 ± 0.88	2.88 ± 0.52	0.83 ± 0.29
400 mg	3	42.8 ± 2.47	4.58 ± 0.19	1.5 ± 1.32

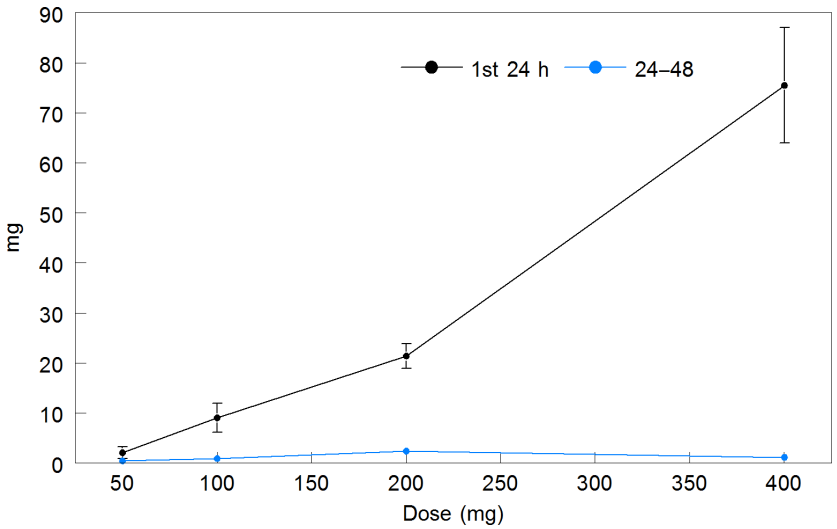

Figure 3. Profile of plasma pharmacokinetics during the first 48 h after a single dose of study drug. Average concentrations with SD error bars. Following single sc injection, ISIS 388626 was rapidly absorbed as demonstrated by reaching maximum plasma concentrations (C_{max}) between 1.2 and 1.5 h.

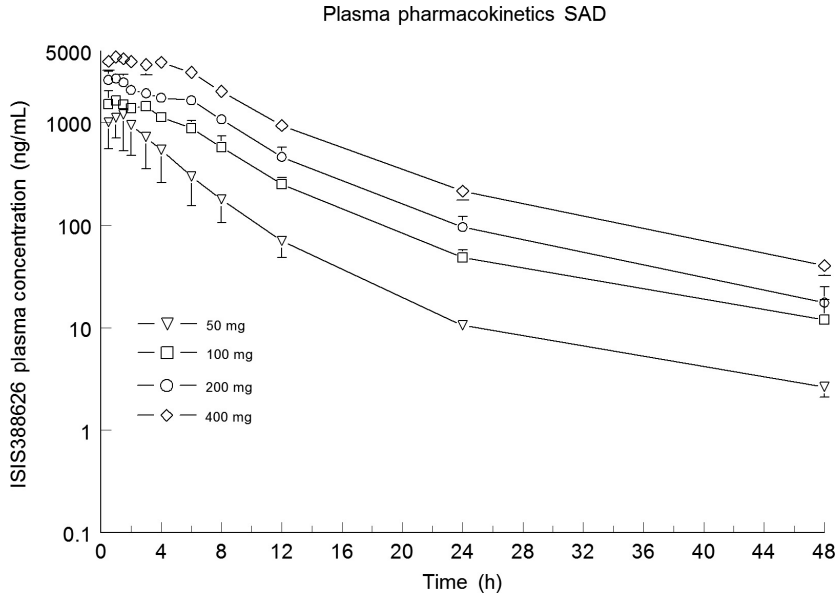

Figure 4. Average ISIS 388626 urinary excretion (mg), with SD error bars after one dose of study drug during the first 24 h and during the 24–48 h interval. *N* = 3 subjects per dose level. The total amount of ISIS 388626 in urine increased more than dose-proportionally

Table 4. Plasma pharmacokinetics multiple ascending dose study.

Dose	Study Day	*N*	AUC 0–48 h (μg*h/mL)	C_{max} (μg/mL)	T_{max} (h)
50 mg	1	6	9.58 ± 1.39	1.65 ± 0.37	1.25 ± 0.27
	36	6	9.78 ± 1.53	1.67 ± 0.26	1.33 ± 0.61
100 mg	1	12	16.1 ± 3.09	2.23 ± 0.36	1.21 ± 1.01

N = number.

did not alter urinary glucose excretion or fractional glucose excretion after 6 weeks of treatment, compared to baseline levels or compared to placebo treatment (Figs. 5 and 6). At follow-up, 6 weeks after the last administration of ISIS 388626, a possible trend toward an increased urinary glucose excretion and increased fractional glucose excretion was observed in the treatment group compared to placebo. However, this observation could not be confirmed by the results of MAD 100 mg cohort, most likely because of the premature study termination.

Discussion

The objectives of these first-in-human study was to assess the safety, pharmacokinetic and pharmacodynamic effects of single and multiple sc doses of ISIS 388626. It was shown that the pharmacokinetics of single doses of the compound was characterized by a rapid absorption after sc administration. Increases in C_{max} and AUC and urinary excretion were almost dose-linear as expected for 20-mer antisense oligonucleotides of this chemical class (Yu et al. 2007; Kastelein et al. 2006; Sewell et al. 2002). Single doses were not associated with any untoward effects.

The multiple dose part of the study was prematurely halted because unexpected findings regarding creatinine increases. However, this part of the study did show that the concept of SGLT2 inhibition by antisense ONs may be promising. The limited data that could be collected showed that antisense-mediated SGLT2 inhibition may result in glucosuria without hypoglycemia. This is in accordance with findings in small molecule SGLT2 inhibitors (Shah et al. 2012) and genetic disruption/absence of SGLT2 gene (Francis et al. 2004).

The unexpected renal findings demonstrated by the substantial increase in serum creatinine and increase in urinary excretion of B2M, aGST, and KIM1 obviously warrant further evaluation. Although the increase in serum creatinine levels could be explained by increased production, this is unlikely because CPK levels in blood and urinary creatinine excretion did not differ significantly between groups (data not shown). This suggests that reduced kidney clearance, based on decreased filtration or decreased tubular secretion of creatinine are likely explanations for our observations. Creatinine clearance is likely to be affected, however, this was not detectable, possibly due to a large inter- and intrasubject variability of creatinine clearance.

The dose-dependent increase in urinary excretion of B2M further supports the view that tubular dysfunction explains our findings. This type of tubular dysfunction

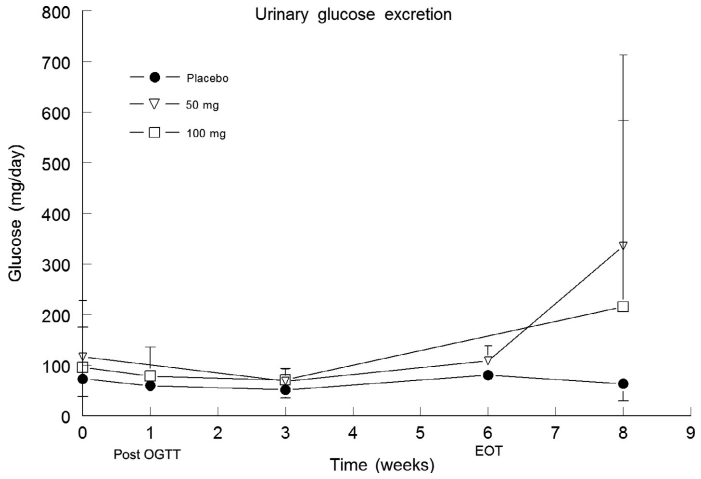

Figure 5. Average urinary glucose excretion time profile graph, with SD error bars. Repeated administration of 50 mg ISIS 388626 did not alter urinary glucose excretion after 6 weeks of treatment. At follow up, six weeks after the last administration of ISIS 388626, a possible trend towards an increased urinary glucose excretion was observed. EOT, End Of Trial; OGTT, Oral Glucose Tolerance Test.

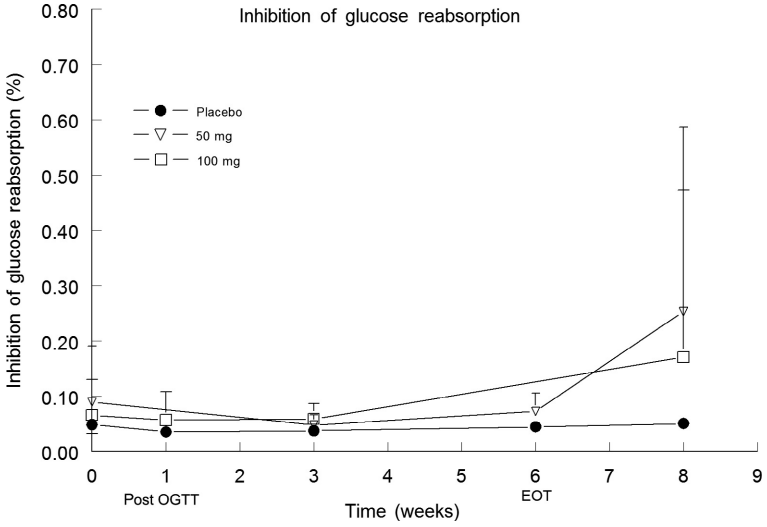

Figure 6. Average inhibition of glucose reabsorption time profile graph, with SD error bars. Repeated administration of 50 mg ISIS 388626 did not alter inhibition of glucose reabsorption after 6 weeks of treatment. At follow-up, six weeks after the last administration of ISIS 388626, a possible trend towards an increased inhibition of reabsorption was observed. W,week; EOT, end of Trial; OGTT, Oral Glucose Tolerance Test.

has been described also for cimetidine and is thought to reflect tubular adaptation to altered function, whereby impaired tubular creatinine secretion results in decreased reabsorption of proteins such as albumin and B2M. This is strengthened by the observation that B2M is filtrated and reabsorbed by proximal tubular cells via similar pathways as luminal uptake of antisense oligonucleotides (Rappaport et al. 1995; Hoffmann et al. 2010). It may be hypothesized that ONs impair megalin- and cubulin-mediated uptake. However, it cannot be ruled out that our findings are explained by tubular damage rather than functional adaptation, as increases of urinary KIM1 and aGST, the latter seemingly related to cumulative dose, were also observed. KIM1 is a transmembrane protein expressed by tubular epithelial cells in response to injury and acts by increasing the capacity to clear cell debris by phagocytosis. Similarly, the increase in urinary excretion of aGST, an intracellular lysosomal enzyme not filtrated by glomerulus, may reflect damage of the organelles of tubular cells. It remains unknown if this is related to the accumulation of oligonucleotide within phagolysosomes of proximal tubule cells, which is a common microscopic finding after oligonucleotide administration in animals (Monteith and Levin 1999; Rappaport et al. 1995). For the majority of ONs this commonly is benign, although there seem to be a few exceptions. Animal studies with

other phosphorothioate ONs have shown an association with proximal tubular degeneration and necrosis in the kidneys (Henry et al. 1997; Sarmiento et al. 1994; Srinivasan and Iversen 1995). This renal toxicity commonly occurs only at doses that are at least 10- to 100-fold higher than administered in clinical trials, therefore, the relevance of these findings for humans is unknown. Whether accumulation of ONs in tubular cells is innocuous when the target is also in the tubuli should be explored further. Thus, tubular dysfunction appears to be a reasonable explanation for our findings. We consider it unlikely that extensive tubular degeneration and necrosis occurred in our study as the changes in renal function were rapidly reversible. It is more likely that a milder form of tubular dysfunction occurred in which the tubuli, which had taken up the ON, showed functional impairment in secreting creatinine and a decreased capacity to reabsorb B2M. A third explanation of the observed signals could be temporary disruption of membrane function. Loss of the SGLT2 transporter could impact expression or function of other transporters in the proximal tubules, needed to maintain the complex balance of apical and basolateral transport. The changes observed were relatively mild and reversible of nature, nonetheless could well reflect sublethal cellular injury taking into account the KIM1 increase.

Due to the early halt adequate assessment of the pharmacodynamic properties of ISIS 388626 was not possible. It appears that there was a trend toward higher urinary glucose excretion during follow-up after 6 weeks of treatment with 50 mg as compared to placebo, but the variability between subjects precludes strong conclusions. The doses and duration of treatment with ISIS 388626 should be increased in future clinical studies in order to achieve exposure comparable with pharmacological effective doses in animals (Zanardi et al. 2012). Obviously, this can only be justified with a better understanding and possibly avoidance of the unexpected adverse renal effects. In this respect, it should be noted that similar effects after comparable treatment regimens with ISIS 388626 in rodents and monkeys did not lead to these similar changes. This may be explained by the fact that sampling time points in these studies were taken after 6 and 13 weeks of dosing (Bhanot 2009). The observed creatinine increases in our study occurred earlier in time and reduced with continued dosing (in the 50 mg cohort). If in animals the renal changes also occurred early, these changes apparently recovered.

More extensive preclinical investigation, focused on early changes and comparing different dose regimens such as abandoning a loading regimen and/or less frequent dosing, could be considered as further experiments. Also, it might be of interest to include in such experiments other drugs that increase serum creatinine, such as cimetidine. Comparing ISIS 388626 to small molecule SGLT2 inhibitors could be used as benchmark and confirm or refute the hypothesis that the renal effects are related to SGLT2 inhibition. Further development of antisense-mediated knockdown of SGLT2 can only be justified when possible mechanisms causing the increase in serum creatinine and urinary excretion of B2M, aGST, and KIM1 are understood and manageable.

Authorship Contribution

Participated in research design: Marloes van Dongen, Adam Cohen, Jacobus Burggraaf. Conducted experiments: Marloes van Dongen, Adam Cohen, Jacobus Burggraaf. Performed data analysis: Leonie van Meer, Marieke de Kam. Wrote or contributed to the writing of the manuscript: Leonie van Meer, Matthijs Moerland, Adam Cohen, Jacobus Burggraaf.

Disclosure

None declared.

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