Pharmacodynamics and Subchronic Toxicity in Mice and Monkeys of ISIS 388626, a Second-Generation Antisense Oligonucleotide That Targets Human Sodium Glucose Cotransporter 2*S

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ABSTRACT

ISIS 388626, a 2′-methoxyethyl (MOE)-modified antisense oligonucleotide (ASO) that targets human sodium glucose cotransporter 2 (SGLT2) mRNA, is in clinical trials for the management of diabetes. SGLT2 plays a pivotal role in renal glucose reabsorption, and inhibition of SGLT2 is anticipated to reduce hyperglycemia in diabetic subjects by increasing urinary glucose elimination. To selectively inhibit SGLT2 in the kidney, ISIS 388626 was designed as a “shortmer” ASO, consisting of only 12 nucleotides with two 2′-MOE-modified nucleotides at the termini. Mice and monkeys received up to 30 mg/kg/week ISIS 388626 via subcutaneous injection for 6 or 13 weeks. Dose-dependent decreases in renal SGLT2 mRNA expression were observed, which correlated with dose-related increases in glucosuria without concomitant hypoglycemia. There were no histologic changes in the kidney attributed to SGLT2 inhibition after 6 or 13 weeks of treatment. The remaining changes observed in these studies were typical of those produced in these species by the administration of oligonucleotides, correlated with high doses of ISIS 388626, and were unrelated to the inhibition of SGLT2 expression. The kidney contained the highest concentration of ISIS 388626, and dose-dependent basophilic granule accumulation in tubular epithelial cells of the kidney, which is evidence of oligonucleotide accumulation in these cells, was the only histologic change identified. No changes in kidney function were observed. These results revealed only readily reversible changes after the administration of ISIS 388626 and support the continued investigation of the safety and efficacy of ISIS 388626 in human trials.

Introduction

The low-affinity/high-capacity sodium glucose cotransporter 2 (SGLT2) plays a pivotal role in renal glucose reabsorption and is an attractive therapeutic target for the treatment of type 2 diabetes (Bailey, 2011). SGLT2 is expressed at the luminal (brush border) membrane of the S1 and S2 segments of the proximal renal tubule. SGLT2 is believed to mediate >80% of renal glucose reabsorption (Kanai et al., 1994; Wood and Trayhurn, 2003). In contrast, the high-affinity, low-capacity glucose transporter SGLT1 is thought to be responsible for 10 to 20% of glucose reabsorption that occurs in the kidney. In cases of hyperglycemia, the inhibition of SGLT2 results in glucosuria and a reduction in serum glucose concentrations. Because this mechanism is independent of either the amount of circulating insulin or changes in insulin sensitivity, it has the potential to be effective in all stages of type 2 diabetes, including in those patients who are refractory to other antidiabetic therapies. The reduction in plasma glucose concentrations after SGLT2 inhibition can also result in secondary improvements in insulin sensitivity and secretion, because of a reduction in glucotoxicity (Fujimori et al., 2008; Han et al., 2008). In addition, because this approach does not involve the alteration of counter-regulatory mechanisms, it is not expected to cause hypoglycemia.

ISIS 388626 [2′-(2-methoxyethyl)-P-thioguanylyl-(3′-O-5′)]-2′-(2-methoxyethyl)-P-thioguanylyl-(3′-O-5′)-2′-deoxy-5-methyl-P-thiocytidyl)-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-P-thiocytmidylyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-P-thiocytmidylyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)-2′-deoxy-P-thioguanylyl-(3′-O-5′)-2′-deoxy-P-thiodenyl-(3′-O-5′)

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ABBREVIATIONS: SGLT, sodium glucose cotransporter; ASO, antisense oligonucleotide; MOE, methoxyethyl; KIT, Korea Institute of Toxicology; BLQ, below the lower limit of quantitation; AUC, area under the curve; CV, coefficient of variation.
Oligodeoxynucleotide Characteristics and Preparation. ISIS 388626 is the sodium salt of a 12-base phosphorothioate oligonucleotide with the sequence 5′-GCGATGACTGC-3′ (molecular formula = C_{132}H_{167}N_{45}O_{68}P_{11}S_{11}Na_{11}; molecular weight = 4418.30 amu). Each of the 11 internucleotide linkages is a 3′-O to 5′-O phosphorothioate diester. Eight of the 12 sugar residues are 2-deoxy-d-ribose, and the remaining four are 2′-(2-methoxymethyl)-d-ribose. The residues are arranged such that two MOE nucleosides at the 5′ and 3′ ends of the molecule flank a gap of eight 2′-deoxycytosides. Each of the three cytosine bases is methylated at the 5-position. ISIS 388626 targets exon 9 of human mRNA for SGLT2 and is homologous to SGLT2 mRNA in multiple species, including mouse, rat, monkey, and human. The oligonucleotide was synthesized at Isis Pharmaceuticals, Inc., by using an OligoProcess II synthesizer (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) with solid-phase phosphoramidite chemistry (Beaucage and Iyer, 1992).

ISIS 388626 was formulated in phosphate-buffered saline at concentrations such that the appropriate amount of the test agent was delivered in a subcutaneous dose of 10 ml/kg body weight to mice and 4 ml/kg body weight to monkeys. In monkeys subcutaneous injection sites were rotated between the interior and exterior aspects of each thigh.

Animals, Husbandry, and Experimental Design. A total of 206 male and 98 female 8-week-old Crl:CD1(ICR) mice ( Orient Bio Inc., Gyeonggi-Do, Korea) and 38 2- to 5-year-old cynomolgus monkeys of each sex (Guangxi Grandforest Scientific Primate Company, Ltd., Dayiling Ping Nan County Guangxi, China) were used for these studies. Animals of both species were individually housed under conditions of controlled light, temperature, humidity, and room air circulation. Monkeys were acclimated to laboratory conditions during which time they were accustomed to chair restraints, which were used during electrocardiography and physical examinations. Animal studies were conducted at the Korea Institute of Toxicology (KIT) in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and studies were approved by the KIT Institutional Animal Care and Use Committee before study initiation.

Main study and toxicokinetic mice and monkeys were randomized to the experimental groups detailed in Table 1 and administered ISIS 388626 or vehicle. The initial week of each study served as a “loading period” during which animals received subcutaneous doses (0, 1, 3, 10, or 30 mg/kg) every other day. Thereafter, they were administered once-weekly subcutaneous injections of 0, 1, 3, 10, or 30 mg/kg for 12 consecutive weeks.

Male mice designated for toxicokinetic analyses were similarly administered 3 or 10 mg/kg/week of ISIS 388626 until their utilization for assessment of plasma kinetics and tissue distribution on experimental days 1 or 42. A single group of monkeys (seven of each sex) was designated for toxicokinetic analyses. Each was administered 3 mg/kg every other day during the 7-day loading period, then they were sacrificed (1/sex/interval) for tissue collection on experimental days 3, 9, 15, 23, 31, 39, and 55.

Toxicokinetic Analyses. Plasma concentrations of ISIS 388626 were determined at multiple time points after administration of the drug to mice and monkeys (detailed in Table 1). Plasma samples were analyzed by using a hybridization enzyme-linked immunosorbent assay (Yu et al., 2002). Tissues from both species were collected and analyzed for ISIS 388626 concentrations by using a validated high-performance liquid chromatography-tandem mass spectrometry method (Murphy et al., 2005). Plasma sample analyses were conducted at KIT, and tissue sample analyses were conducted at PPD Development (Richmond, VA). Plasma and tissue sample analyses were performed based on the principles and requirements as indicated by the Food and Drug Administration (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58&showFR=1). The lower limits of quantitation were 1.00 ng/ml and 0.200 μg/g in plasma and tissue, respectively.

ISIS 388626 plasma concentration-time data were analyzed by noncompartmental (individual animal profiles) methods by using the computer program WinNonlin Professional, version 5.2 (Pharsight, Mountain View, CA). The maximal observed drug concentration (C_{max}) and the time taken to reach C_{max} (t_{max}) were obtained directly from the concentration-time data. The plasma disposition half-life (t_{1/2α}) associated with the initial absorption/distribution phase was calculated by using a noncompartmental analysis extravascular model (WinNonlin) for all of the individual profiles in all treated animals, using the equation t_{1/2α} = 0.693/α, where α is the rate constant associated with the initial absorption/distribution phase. The plasma disposition half-life (t_{1/2α}) associated with the terminal elimination phase was calculated from recovery animals (groups 4 and 5) by using the equation t_{1/2α} = 0.693/λ_{α}, where λ_{α} is the rate constant associated with the terminal elimination phase. A minimum of three data points was used to define the rate constants (α or λ_{α}), and the correlation of determination values had to be more than 0.8 for the estimate to be accepted. Area under the plasma concentration-time curve from zero time (predose) to the 48-h time point (AUC_{0-48h}) or from zero time (predose) to 168 h (dosing interval, τ) (AUC_{0-168}) at steady state was calculated by using the linear trapezoidal rule.
Inhibition of SGLT2 by ISIS 388626 in Mice and Monkeys

TABLE 1
Protocol outline and animal disposition for 13-week toxicity studies of ISIS 388626 in male and female CD-1 mice and cynomolgus monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>Number Male/Female</th>
<th>Dose</th>
<th>Dose Regimen\textsuperscript{a}</th>
<th>Number of Animals Sacrificed, Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Interim</td>
</tr>
<tr>
<td>Mice: main study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22/22</td>
<td>0</td>
<td>q2d/q1w</td>
<td>6/6</td>
</tr>
<tr>
<td>2</td>
<td>16/16</td>
<td>1</td>
<td>q2d/q1w</td>
<td>6/6</td>
</tr>
<tr>
<td>3</td>
<td>22/22</td>
<td>3</td>
<td>q2d/q1w</td>
<td>6/6</td>
</tr>
<tr>
<td>4</td>
<td>16/16</td>
<td>10</td>
<td>q2d/q1w</td>
<td>6/6</td>
</tr>
<tr>
<td>5</td>
<td>22/22</td>
<td>30</td>
<td>q2d/q1w</td>
<td>6/6</td>
</tr>
<tr>
<td>Mice: toxicokinetics</td>
<td>6</td>
<td>54/0</td>
<td>3</td>
<td>3/group at 15 and 30 min and 1, 2, 4, 8, 24, and 48 h after dosing on days 1 and 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>54/0</td>
<td>3/group at 15 and 30 min and 1, 2, 4, 8, 24, and 48 h after dosing on days 1 and 42</td>
</tr>
<tr>
<td>Monkeys: main study</td>
<td>1</td>
<td>7/7</td>
<td>0</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5/5</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5/5</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7/7</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>7/7</td>
<td>2/2</td>
</tr>
<tr>
<td>Monkeys: toxicokinetics</td>
<td>6</td>
<td>7/7</td>
<td>3</td>
<td>1/sex/interval on days 3, 9, 15, 23, 31, 39, and 55</td>
</tr>
</tbody>
</table>

\textsuperscript{a}ISIS 388626 was administered by subcutaneous injection every other day (q2d) for the first 7 days (days 1, 3, 5, and 7) then once weekly (q1w) for the remainder of the study.

Pharmacologic Demonstration. The effect of ISIS 388626 on expression of renal SGLT2 mRNA were assessed in kidney samples collected during interim (week 6), terminal (week 13), and recovery phases in both species. Approximately 100 mg of kidney tissue was homogenized in guanidinium isothiocyanate. Total RNA was then centrifuged over a cesium chloride gradient and resuspended in RNase-free water. Total RNA was purified further by using a RNAeasy mini RNA preparation kit (Qiagen, Valencia, CA). After quantitation, the kidney tissue was subjected to quantitative polymerase chain reaction analysis (Bustin, 2000; Nolan et al., 2006; VanGuilder et al., 2008). The Invitrogen-ABI StepOnePlus real-time polymerase chain reaction system (Invitrogen, Carlsbad, CA) was used. The assay is based on a target-specific probe labeled with a fluorescent reporter and quencher dyes at opposite ends. The probe is hydrolyzed through the 5’exonuclease activity of the Taq DNA polymerase, leading to an increasing fluorescence emission of the reporter dye that can be detected during the reaction. SGLT2 mRNA was then normalized to total RNA as determined by RiboGreen fluorescence from the same RNA sample.

Determination of ED\textsubscript{50} and EC\textsubscript{50}. ISIS 388626 pharmacodynamic (percentage of inhibition of SGLT2 in kidney tissue) data were analyzed by using the computer program Phoenix WinNonlin, version 6.1 (Pharsight). The effective dose to achieve 50% inhibition of SGLT2 mRNA expression in the kidney (ED\textsubscript{50}) and the effective concentration of ISIS 388626 in the kidney to achieve 50% inhibition of SGLT2 mRNA (EC\textsubscript{50}) were calculated by using the Inhibitory Effect Sigmoid E0 models (model 107; Pharsight). The effective dose to achieve 50% inhibition of renal SGLT2 mRNA (EC\textsubscript{50}) were calculated by using the Inhibitory Effect Sigmoid E0 models (model 107; Pharsight).

Toxicology Assessments. Antemortem evaluative criteria for both species included survival, clinical signs, body weight and food consumption, ophthalmological examinations, clinical pathology (hematology, clinical chemistry, and urinalysis in both species and complement analysis in monkeys) and toxicokinetic analyses. Monkeys also received ECG and physical (heart rate and blood pressure) examinations before initiation of dosing, after the first dose, and after completion of weeks 6 and 13. ECG tracings were examined for qualitative abnormalities, QT and RR intervals were determined, and QTc values were derived. The conduct and data collection for all routine observations and physical and clinical examinations was accomplished in compliance with the principles of Good Laboratory Practices according to the standard operating procedures established at the performing laboratory.

Sacrifice, necropsy, and tissue collection for microscopic examinations of main-study mice and monkeys at the interim, terminal, and recovery phases were conducted on study days 44, 93, and 182, respectively. The numbers of animals sacrificed on specific study days are shown in Table 1. Animals were fasted overnight, taken to deep anesthesia with isoflurane or thiopental, then sacrificed by exsanguination.

Grossly observable lesions, injection sites, and representative sections of approximately 45 to 50 organs/tissues were collected from each animal. Testes were fixed in Bouin’s solution, and eyes and optic nerves were fixed in Davidson’s fixative. All other tissue samples were preserved in neutral buffered formalin. Specimens were routinely processed, and the full range of tissues was examined microscopically.

Results

Systemic Exposure of Mice and Monkeys to ISIS 388626. Toxicokinetic analyses verified systemic exposures of mice and monkeys to ISIS 388626 (Tables 2 and 3) and revealed similar toxicokinetic profiles in both species. There were no gender-related differences in monkeys, and data for

<table>
<thead>
<tr>
<th>Subcutaneous Dose</th>
<th>C\textsubscript{max} (\mu g/ml)</th>
<th>T\textsubscript{max} (h)</th>
<th>AUC\textsubscript{0-24} (\mu g h/ml)</th>
<th>T\textsubscript{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>1.52 ± 0.17</td>
<td>0.25</td>
<td>4.25</td>
<td>1.30</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>7.14 ± 1.0</td>
<td>0.25</td>
<td>11.8</td>
<td>1.56</td>
</tr>
<tr>
<td>Day 42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>1.81 ± 0.09</td>
<td>0.25</td>
<td>4.75</td>
<td>1.26</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>6.80 ± 4.2</td>
<td>0.25</td>
<td>15.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are means of n = 3 ± S.D.
that species were pooled across the sexes to simplify graphic and tabular representation.

ISIS 388626 was readily absorbed from subcutaneous administration sites with $C_{\text{max}}$ values being attained in 0.25 h in mice and 0.6 to 1.5 h in monkeys and then decreased in an apparent multieponential fashion with time. Systemic exposure, as exemplified by AUC values, increased with dose, and in mice the magnitudes of increments between the 3 and 10 mg/kg doses were generally dose-proportional. In monkeys, increments between AUC values achieved after 1 and 3 mg/kg were consistently dose proportional as were increments between 3 and 10 mg/kg doses. Increments between AUC values achieved after 1 and 3 mg/kg were somewhat supraproportional as were increments between 10 and 30 mg/kg. Increments between 3 and 10 mg/kg were somewhat supraproportional to dose.

Elimination of ISIS 399626 from the plasma (evidenced by distribution half-life, $T_{1/2}$) ranged from 1 to slightly longer than 3 h and was attributable mainly to distribution to and storage in peripheral tissues, primarily the kidney (Yu et al., 2009). The estimated mean terminal elimination plasma half-life values ($t_{1/2\alpha}$) obtained from recovery animals were 15.3 and 22.2 days after 10 and 30 mg/kg/week dose for 13 weeks, respectively.

Plasma ISIS 388626 concentration curves for the two species were similar (Figs. 1 and 2). Consistent with toxicokinetic evidence of systemic exposure in mice, day 1 and day 42 plasma concentration curves within doses were indistinguishable, and there was no evidence of bioaccumulation of the drug in mouse plasma but there was some evidence of accumulation in monkey plasma on days 42 and 91 relative to day 1 at 48 h. Although increments in $C_{\text{max}}$ values in mice were somewhat supraproportional to dose on both days 1 and 42 (Table 2), and there was no evidence of bioaccumulation in plasma with concentrations on days 1 and 42 being indistinguishable.

After subcutaneous injection ISIS 388626 was distributed primarily to the kidneys in mice and monkeys. Liver contained measurable concentrations of ISIS 388626; however, the concentrations in liver were approximately 5- to 100-fold lower than the concentrations in the kidneys in both mice and monkeys (Table 4). Concentrations of intact ISIS 388626 in kidney of mice and monkeys were dose-dependent, but the increase was less than dose-proportional over the dose range studied. Concentrations of ISIS 388626 in kidney of mice and monkeys increased approximately 3- to 4-fold over a 30-fold increase in dose, which suggests saturation of kidney uptake (Table 4). Meanwhile, concentrations of ISIS 388626 in liver of mice were approximately dose-proportional, and for monkeys they were greater than dose-proportional over the dose range studied. Tissue concentrations of ISIS 388626 after 13 weeks of treatment were approximately 5- to 20-fold higher in monkeys than in mice at comparable milligram/kilogram doses (Table 4).

Elimination of ISIS 388626 from tissues was slow. The tissue half-lives of intact ISIS 388626 (12-mer) were 6 and 7 days in monkey kidney and liver, respectively. Although slow, elimination of ISIS 388626 from tissues was also observed during the recovery period (day 181). Concentrations of oligonucleotide in the tissues of animals assigned to the recovery group were substantially lower than those measured in monkeys that had been necropsied 2 days after the last dose (day 93). For example, concentrations of ISIS 388626 remaining in kidney cortex and liver after the treatment-free period were approximately 0.5 to 5% of the concentrations at the end of treatment.

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Level</th>
<th>Study Day</th>
<th>$T_{\text{max}}$</th>
<th>$C_{\text{max}}$</th>
<th>AUC$_{0-48,\text{h}}$</th>
<th>AUC$_{0-168,\text{h}}$</th>
<th>$t_{1/2\alpha}$</th>
<th>$t_{1/2\alpha}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10 mg/kg</td>
<td>42</td>
<td>0.6 ± 0.2</td>
<td>1.74 ± 0.27</td>
<td>5.92 ± 0.41</td>
<td>N.M.</td>
<td>1.91 ± 0.25</td>
<td>N.M.</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>42</td>
<td>1.1 ± 0.7</td>
<td>2.38 ± 0.77</td>
<td>7.19 ± 1.72</td>
<td>7.09 ± 1.42</td>
<td>2.03 ± 0.23</td>
<td>N.M.</td>
</tr>
<tr>
<td>4</td>
<td>10 mg/kg</td>
<td>91</td>
<td>1.4 ± 0.5</td>
<td>2.06 ± 0.53</td>
<td>9.56 ± 0.35</td>
<td>N.M.</td>
<td>1.9 ± 0.09</td>
<td>N.M.</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>42</td>
<td>1.4 ± 0.5</td>
<td>1.91 ± 0.47</td>
<td>10.17 ± 2.12</td>
<td>N.M.</td>
<td>1.91 ± 0.09</td>
<td>N.M.</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>42</td>
<td>1.4 ± 0.5</td>
<td>1.91 ± 0.47</td>
<td>10.17 ± 2.12</td>
<td>N.M.</td>
<td>1.91 ± 0.09</td>
<td>N.M.</td>
</tr>
</tbody>
</table>

$t_{1/2\alpha}$, general estimate of plasma half-life associated with the initial (major) distribution phase; $t_{1/2\alpha}$, the plasma disposition half-life associated with the terminal elimination phase was calculated from recovery animals (groups 4 and 5). N.M., not measured.
Inhibition of SGLT2 by ISIS 388626 in Mice and Monkeys

Pharmacologic Effects. Both mice and monkeys exhibited dose-dependent decreases in renal SGLT2 mRNA expression reflecting the intended pharmacologic activity of the drug. SGLT2 mRNA was reduced by 74 to 97% (26 to 3% of control) in mice and by approximately 30 to 90% (70 to 10% of control) in monkeys over the dose range tested (1–30 mg/kg/week; Figs. 3A and 4A, respectively). The magnitudes of inhibition in each species were comparable after the 6- and 13-week treatment regimens. During the treatment-free recovery phase renal SGLT2 expression returned to approximately 70 and 50% of control at 30 mg/kg/week in mice and by approximately 30 to 90% (70 to 10% of control) in monkeys over the dose range tested (1–30 mg/kg/week; Figs. 3A and 4A, respectively). The magnitudes of inhibition in each species were comparable after the 6- and 13-week treatment regimens. 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Administration of \(10\) mg/kg/week to mice caused a slight (1.1- to 1.3-fold) increase in mean kidney and spleen weights in both sexes (Supplemental Table 3). These small changes were corroborated by similar changes in relative (to brain and body) organ weight parameters. After the 13-week recovery period mean kidney and spleen weights in dosed mice were indistinguishable from controls. ISIS 388626 had no effect on absolute or relative organ weights in monkeys.

Treatment-related microscopic changes in interim (6
weeks) sacrificed mice and monkeys were limited to animals administered \(10\) mg/kg/week and reflected the uptake and accumulation of the oligonucleotide at peripheral organ storage depots (Henry et al., 2008, 2012). An interim change in mice, hepatic centrilobular hypertrophy, noted in 1/3 males

### Table 4
ISIS 388626 concentrations in tissues measured approximately 2 Days after the last dose (day 93) after 13 weeks of repeated dose administration and 13 weeks of recovery (day 182) in mice and monkeys

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day</th>
<th>Kidney</th>
<th>Liver</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg</td>
<td>93</td>
<td>21.5 ± 10.9</td>
<td>0.37 ± 0.057</td>
<td>152 ± 73</td>
<td>2.19 ± 0.54</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>93</td>
<td>32.2 ± 12.8</td>
<td>0.82 ± 0.156</td>
<td>236 ± 91</td>
<td>10.3 ± 3.3</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>182</td>
<td>BLQ</td>
<td>BLQ</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>182</td>
<td>43.6 ± 13.2</td>
<td>2.56 ± 0.58</td>
<td>414 ± 134</td>
<td>62 ± 20.7</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.69 ± 1.33</td>
<td>0.333 ± 0.391</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>58.6 ± 24.3</td>
<td>5.97 ± 1.81</td>
<td>658 ± 243</td>
<td>162 ± 58</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>BLQ</td>
<td>BLQ</td>
<td>4.18 ± 2.28</td>
<td>7.81 ± 6.04</td>
</tr>
</tbody>
</table>

BLQ indicates mean concentrations that were below the lower limit of quantitation, which was 0.2 \(\mu g/g\). N.D., not determined.

### Figure 3
SGLT2 mRNA reduction in kidney (A) and urine glucose excretion (B) in male and female (sexes combined) mice administered ISIS 388626 for up to 13 weeks then afforded a 13-week recovery period. Error bars represent S.D. \((n = 6\) for A; \(n = 3\) for B.

### Figure 4
SGLT2 mRNA reduction in kidney (A) and urine glucose excretion (B) in male and female (sexes combined) cynomolgus monkeys administered ISIS 388626 for up to 13 weeks then afforded a 13-week recovery period. Error bars represent S.D. \((n = 4\) at 6 weeks and 6 at 13 weeks for A; \(n = 14\) at 6 weeks, 10 at 13 weeks, and 4 at recovery for B.)
dosed with 10 mg/kg/week and 3/3 males administered 30 mg/kg/week, was characterized by enlarged hepatocytes in the centrilobular zone. Treatment-related microscopic changes in monkeys (Supplemental Table 5) administered 30 mg/kg/week ISIS 388626 for 6 weeks included Kupffer cell accumulation of basophilic granules (2/2 animals of each sex), histiocytic hypertrophy and vacuolation of lymph nodes (2/2 of each sex), and basophilic granules deposited in renal tubular epithelium (1/2 females). Thirteen consecutive weeks of treatment of mice with 10 or 30 mg/kg ISIS 388626 resulted in additional deposition of basophilic granules in kidney proximal tubular epithelial cells and liver Kupffer cells, and centrilobular hypertrophy was again observed at ≥10 mg/kg/week (Supplemental Table 4). Additional microscopic changes, indicative of a proinflammatory effect, included minimal to moderate splenic extramedullary hematopoiesis in all groups (with a slight increase in severity with increasing doses) and minimal to slight sinus histiocytosis and lymphoid hyperplasia in multiple lymph nodes (mandibular, inguinal, and mesenteric) at doses ≥10 mg/kg/week, with slightly higher incidence and/or severity in males. Minimal lymphohistiocytic infiltration was observed in multiple organs and tissues (kidney, liver, heart, spleen, tongue, lung, esophagus, uterus, vagina, urinary bladder, skin/mammary gland, and injection site).

Terminally sacrificed monkeys exhibited only microscopic changes reflective of uptake and accumulation of the oligonucleotide in peripheral tissues. Changes were qualitatively and quantitatively similar in both sexes and were confined to the kidneys, liver, lymph nodes, and urinary bladder. Changes in terminally sacrificed monkey kidneys were minimal to moderate basophilic granular accumulations in the cytoplasm of tubular epithelial cells in the outer cortex at ≥10 mg/kg/week. Hepatic changes were limited to minimal to slight accumulation of basophilic granules in Kupffer cells with hyperplasia and/or hypertrophy of Kupffer cells at ≥10 mg/kg/week. In the mandibular, mesenteric, and inguinal lymph nodes, minimal to moderate hypertrophy of histiocytes (in one or more animals at 10 and 30 mg/kg/week ISIS 388626) and minimal basophilic granule accumulation and vacuolation in the cytoplasm of histiocyte (in one or more animals at 10 and 30 mg/kg/week ISIS 388626) were observed. Minimal submucosal histiocytic hypertrophy was observed in urinary bladder at 30 mg/kg/week ISIS 388626.

After the recovery period, treatment-related histopathologic changes in mice were partially resolved as evidenced by decreased incidences and/or severities after the 13-week treatment-free period. Minimal changes in the animals recovering from the 30 mg/kg/week regimen included basophilic granules in the kidneys and liver, sinus histiocytosis in the mandibular and mesenteric lymph nodes, and lymphohistiocytic infiltration in kidney, liver, esophagus, skin/mammary gland, and injection sites. In monkeys all ISIS 388626-related microscopic changes were resolved after the treatment-free period.

**Discussion**

Subcutaneous administrations of ISIS 388626 to mice and monkeys resulted in extensive and dose-related systemic exposure to the drug. Absorption from subcutaneous injection sites was prompt with maximal plasma concentrations being achieved within 15 min (mice) to 90 min (monkeys). Plasma concentration curves for both species reflect rapid elimination from plasma. There was no evidence of accumulation of ISIS 388626 in plasma during the once-weekly dosage regimen. However, the long residence time in tissues, kidneys in particular for this particular target, was quite long (T1/2 = approximately 7 days), and supports the infrequent weekly dose regimen used in these studies to produce a sustained inhibition of SGLT2 mRNA expression.

Rapid elimination of drug from the plasma, featuring distribution to peripheral organs with little urinary excretion, is characteristic of both first- and second-generation antisense oligonucleotides (Henry et al., 2001, 2008). The target for ISIS 388626 (SGLT2) resides in the kidney rather than the liver where most targets for 2'-MOE oligonucleotides have been located. Therefore, ISIS 388626 was designed as a short oligonucleotide (12 nucleotides long versus 20 nucleotides for most ASOs) to optimize distribution to the renal proximal tubular epithelium. At the conclusion of the 13-week dosing period in this study the greatest concentrations of ISIS 388626 were found in the renal cortex of both species with much lower concentrations in liver and spleen. Still lesser concentrations of ISIS 388626 were found in essentially every organ examined, and no drug was measured in the brain. As is the case with 20-mer ASOs, urinary excretion accounted for only a small percentage of the administered dose (ranging from 12 to 26% over the dose range of 1 to 30 mg/kg in monkeys) within the first 48 h of administration (Yu et al., 2009).

The desired pharmacologic effect of ISIS 388626, inhibition of the expression of SGLT2 in renal proximal tubules, was demonstrated by mRNA expression analysis, which revealed a dose relatedness in both species. After 13 weeks of treatment, SGLT2 expression was reduced by approximately 80 to 97% (20 to 3% of control) in mice, with an ED50 of <1.0 mg/kg/week, and it was reduced by approximately 30 to 90% (70 to 10% of control) in monkeys, with an ED50 of approximately 3.0 mg/kg/week. The reduction of SGLT2 mRNA in both mouse and monkey was accompanied by dose-related increases in urinary excretion of glucose. In mice, urine glucose was increased by approximately 14- to 130-fold over control at 1 to 30 mg/kg/week, and in monkey urine glucose was increased by approximately 7- to 125-fold over control at 3 to 30 mg/kg/week.

Despite the reduction of renal SGLT2 expression and the resulting glucosuria, clinical pathology analyses revealed no evidence of hypoglycemia. It is likely that hepatic metabolic processes maintained euglycemia by compensating for the renal elimination of glucose. However, neither biochemical nor microscopic changes suggestive of ongoing gluconeogenesis and/or glycogenolysis were observed in either species.

Administration of supra pharmacological doses of ISIS 388626 for 13 consecutive weeks elicited no grossly observable signs of systemic toxicity in either mice or monkeys. Increased body weight gains by mice administered 10 or 30 mg/kg/week probably were related to increased food consumption, which could have been precipitated by a period of relative hypoglycemia that may have been undetected during clinical chemistry analyses.

The lack of effect on activated partial thromboplastin time or alternative complement pathway activation in the monkey (Henry et al., 1997a,b; Sheehan and Phan, 2001) probably reflects reduced plasma protein binding as a result of the
relatively short length of ISIS 388626 (12 nucleotides in length) compared with other phosphorothioate ASOs, which are typically 20 nucleotides long (Yu et al., 2007).

Small and reversible hematologic changes observed in mice were typical of those observed in rodents administered large doses of oligonucleotides and were unrelated to the inhibition of SGLT2 expression. Similar hematologic changes were not observed in monkeys despite extensive inhibition of SGLT2 expression. Proinflammatory effects, including increased spleen weight and multiorgan cell infiltrates, are commonly observed in mice administered high doses of traditional 20-nucleotide ASOs (Henry et al., 2001, 2008). Although these findings were also observed in mice with ISIS 388626, they occurred with a lower incidence and severity compared with most 2′-MOE ASOs, thus reflecting a lower degree of proinflammatory effect for this relatively shorter-length oligonucleotide. In monkeys, there was essentially no evidence of the proinflammatory effects, including no inflammatory cell infiltrates in the skin of the injection site. The reversibility of serum chemistry changes was proven during the treatment-free period. That these serum chemistry changes are rodent specific and unrelated to the inhibition of SGLT2 expression was substantiated by their absence in monkeys that had been administered the drug and were exhibiting the pharmacologic effects. Although proinflammatory effects are not as prevalent in monkeys treated with ASOs, slight increases in spleen weight and cell infiltrates at the subcutaneous injection site are typically observed at high doses (Henry et al., 2008). The absence of these findings in this study provides further evidence that ISIS 388626 is less proinflammatory than the typical 20-nucleotide ASO.

Tissue deports of ISIS 388626 and other antisense oligonucleotides are visualized microscopically as basophilic granules, which are most prominent in kidney, liver, and spleen (Henry et al., 2008). Specifically, it is the distribution of oligonucleotide to the phagocytically active cells in these organs, such as the proximal convoluted renal tubules and hepatic Kupffer cells, where the drug is processed into endosomes and lysosomes. Upon staining the tissues with hematoxylin, oligonucleotides in the lysosome are visualized microscopically as basophilic granules (Monteith et al., 1999; Henry et al., 2008). Basophilic granules are common nonspecific effects of 2′-MOE ASO that reflect uptake of oligonucleotide and are not considered toxicologically relevant. The extent of basophilic granulation was clearly dose related and resolved after cessation of dosing.

The results presented here indicate that it is possible, through minor modifications such as shortening the length of the ASO (12-mer), to optimize distribution to the kidney to specifically inhibit target expression in the kidney. ISIS 388626 provides a good example of an ASO targeting a human target mRNA that has cross-species activity and also works in the kidney. Robust, dose-dependent mRNA target reduction was observed and also reflected in the intended phenotypic change (glucosuria). Good tolerability of the short oligo was also achieved, with lesser degree of proinflammatory effects and complement activation than typical 20-nucleotide MOE. Furthermore, the subchronic safety assessment in mice and monkeys, combined with the nonclinical pharmacology assessments, provides sufficient justification for progression to human clinical studies to evaluate the safety and efficacy of ISIS 388626 for clinical application.

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Authorship Contributions

Participated in research design: Zanardi, Yu, and Henry.

Conducted experiments: Han, Jeong, and Chakravarty.

Performed data analysis: Zanardi, Rime, Yu, and Chakravarty.

Wrote or contributed to the writing of the manuscript: Zanardi, Yu, and Henry.

References


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