A Novel Allosteric Insulin Receptor–Activating Antibody Reduces Hyperglycemia without Hypoglycemia in Diabetic Cynomolgus Monkeys

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Received September 28, 2015; accepted November 17, 2015

ABSTRACT

XMETA is a fully human, allosteric monoclonal antibody that binds the insulin receptor with high affinity and mimics the glucoregulatory, but not the mitogenic, actions of insulin. Here we evaluated the efficacy of both single and repeat s.c. administrations of XMETA in reducing hyperglycemia in obese cynomolgus monkeys with naturally developed type 2 diabetes, a model that shares many features of human diabetes. The data show that a single s.c. administration of XMETA at dose levels ranging from 1.5 to 10 mg/kg markedly reduced fasting hyperglycemia, with a peak effect occurring 1 to 2 days after administration, and sustained for up to 1 week. XMETA’s effect on hyperglycemia was observed without elevations in serum insulin and was concomitant with reduced serum C-peptide levels, even at the lowest dose. Subchronic effects were evaluated via once weekly s.c. administration of XMETA, 10 mg/kg, for 6 weeks. XMETA treatment resulted in robust weekly decreases in fasting glucose levels averaging approximately 30% throughout the study, along with a significant absolute reduction from the vehicle control baseline of 1.2% in hemoglobin A1c, a marker of long-term glycemic status. XMETA treatment was well tolerated with no injection-site reactions, no body weight gain, and no episodes of clinical hypoglycemia. Thus, XMETA shows acute and subchronic improvements in glycemic control in spontaneously diabetic cynomolgus monkeys with a broad safety margin. This profile supports the development of XMETA as a novel glucose-lowering therapeutic agent for the management of type 2 diabetes.

We have recently described a fully human IgG2 monoclonal antibody, XMETA, which binds with high affinity and selectivity to the insulin receptor (IR) and improves glucose metabolism (Bhaskar et al., 2012). It acts as an allosteric partial agonist of the IR and, hence, does not directly interfere with insulin binding. Moreover, in cultured Chinese hamster ovary cells expressing the human IR, XMETA activates Akt, a key enzyme in insulin’s metabolic action, with minimal activation of the mitogen-activated protein kinase/extracellular signal–related kinase (ERK) pathway linked to insulin’s mitogenic activity (Bhaskar et al., 2012; Bedinger et al., 2015a). Consequently, XMETA effectively promoted glucose uptake in insulin-responsive 3T3 cells, but it does not induce the insulin-sensitive proliferation of cancer cells.

In an insulin-resistant, insulinoopenic mouse model of diabetes (multi-low-dose streptozotocin/high-fat-diet mouse), XMETA given i.p. at 10 mg/kg twice per week for 6 weeks normalized fasting hyperglycemia, reduced elevated blood glucose levels after a glucose challenge (1 g/kg by i.p. injection or by oral gavage), and reduced Hba1c from ~12% to 9%. The treatment also reduced nonfasting glucose and other metabolic indices of diabetes, including non–high-density lipoprotein cholesterol, free-fatty acid levels, and β-hydroxy butyrate, but XMFA did not result in either hypoglycemia or weight gain. XMETA did not result in either hypoglycemia or weight gain.

Abbreviations: Act, protein kinase B; ANOVA, analysis of variance; DIO, diet-induced obesity; ELISA, enzyme-linked immunosorbent assay; FSG, fasting serum glucose; Hba1c, hemoglobin a1c; IR, insulin receptor; NHP, nonhuman primate; T2DM, type 2 diabetes mellitus.
gain in this study (Bhaskar et al., 2012). In hyperinsulinemic mice, with diet-induced obesity (DIO), XMetA given i.p. at 10 mg/kg twice per week for 4 weeks normalized fasting glucose without contributing to weight gain. XMetA treatment also corrected glucose tolerance, improved non–high-density lipoprotein cholesterol, and decreased elevated C-peptide levels in the DIO mice (Bhaskar et al., 2013). Furthermore, recent data indicate that a single s.c. administration of XMetA at 10 mg/kg to insulin-dependent spontaneous type 2 diabetic rhesus monkeys substantially reduced fasting hyperglycemia for several days along with a modest effect on nonfasting hyperglycemia (Zhao et al., 2014). Consistent with the diabetic mice study results, no hypoglycemia was observed in these diabetic monkeys at XMetA doses up to 30 mg/kg. Taken together, these studies indicate that XMetA can modulate IR signaling and improve glucose metabolism in models of diabetes. By mimicking the beneficial actions of insulin with a potentially greater therapeutic index, this antibody may provide a novel approach for glucose control in patients with diabetes as a broad use injectable adjunctive or alternative to current antidiabetic drugs (Ussar et al., 2011; Vigneri et al., 2012; Issafras et al., 2014).

The primary objective of the current study was to evaluate XMetA efficacy and safety in another relevant animal model of type 2 diabetes: spontaneously diabetic obese cynomolgus monkeys (Macaca fascicularis). These animals exhibit a T2DM profile that is quite similar to that of the human disease (Harwood et al., 2012; Wang et al., 2013) and, because the colony was not dependent on exogenous insulin administration, confounding pharmacology was avoided. Accordingly, XMetA was evaluated for short-term efficacy across a range of doses (1.5, 3, 10 mg/kg) and longer-term efficacy at a single dose level (10 mg/kg) via s.c. administration in nine diabetic cynomolgus monkeys. Metabolic endpoints included both blood glucose and fasting serum glucose (FSG), and other parameters including serum insulin, C-peptide, and triglycerides, and whole-blood HbA1c. XMetA drug levels in the serum and generation of an antidrug antibody response were also assessed.

Methods

Antibodies and Reagents

XMetA antibody was generated by XOMA and formulated in a proprietary vehicle containing l-histidine, arginine, and polysorbate 20. XMetA antibody stock solutions in vehicle and the vehicle were stored at −80°C and, once thawed, were stored at 4°C. Dosing solutions were prepared fresh under sterile conditions by diluting XMetA stock solution in vehicle to the appropriate concentration before administration at room temperature. Purity and stability were assured via ongoing drug substance quality monitoring.

Animals

Animal studies were performed at Crown Bioscience Inc. (Taicang, Jiangsu Province, P.R. China) using cynomolgus monkeys (Macaca fascicularis). Nonhuman primate (NHP) care and use were conducted in accordance with all applicable Association for Assessment and Accreditation of Laboratory Animal Care regulations and guidelines. All experimental procedures and handling, care, and treatment of the animals in the study were approved by the Institutional Animal Care and Use Committee of Crown Bioscience and were performed according to guidelines for the Association for Assessment and Accreditation of Laboratory Animal Care. Animals were single-housed and had free access to water and a normal-calorie diet enriched with seasonal fruits. Animals were routinely monitored for any effects of the compound on their behaviors such as mobility, food and water consumption, body weight gain or loss, and any other abnormal activities.

From an initial set of 20 spontaneously diabetic cynomolgus monkeys, nine animals were selected for the current study based on screening results (age, body weight, glucose, insulin, C-peptide, HbA1c). The selected monkeys included eight females and one male, were non-naïve (although naïve to biologics), with variable body weight (3.6–11.2 kg) and age (11–27 years), and had spontaneously developed diabetes (non–insulin-dependent) as indicated by an overnight FSG level of approximately 146–270 mg/dl and HbA1c ≥ 6.3%. The individual data of the monkeys, as well as means and S.E.M. for each parameter are provided in Table 1. Normal cynomolgus monkey FSG and HbA1c levels are 40–75 mg/dl and ≤5%, respectively (Crown Bioscience; historic data). None of the monkeys received exogenously administered insulin.

Animal Study Design

Acute and Subchronic Efficacy Testing. An overview of the study design is presented in Fig. 1. In the first phase, the nine selected animals were acclimated in study housing for a week with baseline measurements. In the following week, animals received s.c. administration of the vehicle on day 1 and of XMetA on day 8 at 11 AM at a dosing volume of 0.3 ml/kg. Five monkeys received 3 mg/kg XMetA on day 8 and the other four 10 mg/kg of XMetA. Subsequently, both groups were then administered 10 mg/kg of XMetA once per week at 11 AM (days 15, 22, 29, 36, 43) for another 5 weeks. During the subchronic efficacy testing, a provision was established wherein animals exhibiting substantially reduced hyperglycemia (i.e., <125 mg/dl) on the morning of dosing could be administered a reduced XMetA dose. Accordingly, one well-responding animal received 5 mg/kg on days 22 and 29 but 10 mg/kg on all other days. Animals were fasted for 12 hours overnight (starting at 7 PM until 7 AM the next day) on days −6, 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, and 46. Clinical observations were conducted twice a day during the treatment period. Body weights were measured on day −5 before treatment, and twice weekly during the treatment period (at the end of fasting periods). Food consumption was measured on 3 consecutive days before start of the treatment and measured daily during the treatment period. Animals were monitored for clinical signs of hypoglycemia; in addition, hypoglycemia was defined as having a serum glucose level lower than 40 mg/dl.

Lower-Dose Acute Efficacy Testing. In a follow-up study in the same monkeys the efficacy of a single, lower, s.c. dose of XMetA to control hyperglycemia was evaluated in four monkeys that previously responded to XMetA and had not developed an immunogenicity response. After a drug washout period of 28 days after the end of the repeat-dose study, these monkeys were s.c. administered vehicle on day −5 at 11 AM and then XMetA on day 1 at 1.5 mg/kg. Animals were fasted for 12 hours overnight (starting at 7 PM until 7 AM the next day) on days −5, 1, and 4. Clinical observations were conducted twice a day, body weights were measured on days −4, 2, and 5 (at end of fasting), and food consumption was measured daily.

Glucose Assessments and Blood Sampling

In the repeat-dose study, glucometer readings for blood glucose measurements were obtained at 7 AM on days −6 and −5 and at 7 PM on day −6 before treatment and three times daily during the treatment period (at 7 AM right after lights on, at 1 PM, and at 7 PM right before lights off), including fasting days. In the follow-up study, glucometer readings were obtained at 7 AM on day −4, and three times daily from days 1–7 (at 7 AM, 1 PM, and 7 PM), including fasting days. Glucometer readings were done using a monkey chair or cage-side via tail-vein prick of the animals using a glucometer from ACCU-CHEK. Animals were trained for the blood sampling procedures to minimize stress. Glucometer readings were performed in duplicate (in triplicate when the two measures differed more than 30 mg/dl) with an upper limit of 600 mg/dl.
Blood was also drawn from each monkey at the end of the 12-hour fasting periods for serum drug level and antidrug antibody analysis and metabolic parameter assays. At each time point, the monkey was chaired and whole blood was collected via a cephalic vein. The animal was returned to its cage immediately after the collection. The pharmacodynamic time points were at 7 AM at the end of each fasting period. Pharmacokinetic time points were at 7 AM on day –5 and twice weekly during the first 2 weeks of treatment. During weeks 3–7 and week 13 (after washout), pharmacokinetic blood draws were performed 5 days/week, at 7 PM on the dosing day, and at 7 AM on the other days. Blood samples were collected into serum separator tubes and kept at 4°C for analysis on the same day.

For measurement of HbA1c, 0.5 ml of whole blood was utilized excessive XMetA as competitor. Briefly, in the screening fashion consisting of screening and a competitive confirmatory assay limit was 19.5 ng/ml. Samples were interpolated from the calibration curve. The detection parameter logistic curve. The concentrations of unknown and control parameter logistic curve. The concentrations of unknown and control calibrators were fitted to a five-parameter logistic curve. The concentrations of unknown and control samples were interpolated from the calibration curve. The detection limit was 19.5 ng/ml.

The evaluation of immunogenicity was carried out in a tiered fashion consisting of screening and a competitive confirmatory assay utilizing excessive XMetA as competitor. Briefly, in the screening

**Metabolic Parameters**

Monkey serum and whole-blood samples were tested at a validated clinical laboratory with methods standardized for clinical measurements. Fasting glucose and triglyceride levels in serum were measured using enzymatic assays, using glucose oxidase (DiaSys, Holzheim, Germany) and GK-PK-LDH (glycerol kinase-pyruvate kinase-lactate dehydrogenase; Siemens Healthcare Diagnostics, Tarrytown, NY) enzymes, respectively, on a Siemens ADVIA-2400 clinical chemistry system. Insulin and C-peptide serum levels were measured using two-site sandwich chemiluminescence immunoassays, the ADVIA Centaur XP Insulin and ADVIA Centaur XP C-peptide assay, respectively (Siemens Healthcare Diagnostics). HbA1c was measured in whole blood samples by ion-exchange high-performance liquid chromatography (HPLC) using the Bio-Rad D-10 hemoglobin A1c system (Bio-Rad, Hercules, CA).

**Measurement of XMetA and Antidrug Antibody Levels in Monkey Serum**

Both XMetA concentrations and titers of anti-XMetA antibodies in cynomolgus monkey serum were measured by sandwich colorimetric enzyme-linked immunosorbent assays (ELISA). The XMetA assay used recombinant human IR (2 µg/ml; R&D Systems, Minneapolis, MN) passively coated onto a Nunc-Immuno Maxisorp 96-well micro-titer plate (Thermo Fisher Scientific, Waltham, MA) as the capture reagent. Plates were incubated overnight at 2–8°C. The following day, after a blocking and washing step, plates were incubated with experimental samples, controls, and calibration standards for 1 hour at room temperature; washed; and incubated with biotinylated goat anti-human IgG (0.125 µg/ml; monkey adsorbed; Southern Biotech, Birmingham, AL) for 1 hour at room temperature. After washing, plates were incubated with streptavidin-conjugated alkaline phosphatase at room temperature for 15 minutes. Finally, plates were incubated in the dark for 1 hour at room temperature with p-nitrophenyl phosphate (PNPP; Thermo Fisher Scientific) in diethanolamine (Thermo Fisher) buffer. The reaction was stopped by addition of 1N NaOH. Plates were read in a Molecular Devices SpectraMax M2 plate reader at 405 nm (Molecular Devices, Sunnyvale, CA). The optical densities versus concentrations of XMetA standard calibrators were fitted to a five-parameter logistic curve. The concentrations of unknown and control samples were interpolated from the calibration curve. The detection limit was 19.5 ng/ml.

Fig. 1. Study design in diabetic cynomolgus monkeys. Animals were administered vehicle or XMetA antibody weekly at 11 AM each Monday in the subchronic (repeat-dose) study. After washout, selected animals received a single low dose of SC XMetA. One animal was excluded from data analysis because of high basal insulin; two animals showed an immunogenicity response, one early and one late in the study. One animal was excluded from data analysis owing to lack of drug exposure confirmed by serum XMetA analysis.

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<th>C-peptide</th>
<th>Glucose&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HbA1c</th>
<th>Insulin</th>
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<td>0.9</td>
<td>16.8</td>
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<sup>a</sup>Fasting serum glucose.

<sup>b</sup>Range based on historical data from Crown Bioscience Inc.

<sup>c</sup>One animal was hyperinsulinemic; without this animal, the mean ± S.E.M. of insulin is 28.1 ± 8.8.

**TABLE 1**

Screening profile of obese type 2 diabetic cynomolgus monkeys

Subchronic Study

<table>
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<th>Vehicle Baseline</th>
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<th>N=5</th>
<th>10 mg/kg</th>
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<td>Week 3-7</td>
<td>Week 12</td>
<td>Week 13</td>
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Low-dose Acute Test

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<tbody>
<tr>
<td>Week 12</td>
<td>Week 13</td>
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</table>
of XMetA. Data Analysis
All data are presented as mean ± S.E.M. Statistical analyses were performed using a repeated-measures analysis of variance (ANOVA) using a linear mixed-effects model and test-control contrast matrix in R (version 3.1.2) or one-way or two-way ANOVA (repeated measures where appropriate) followed by Fisher’s post-hoc tests using Prism 6 graphing software (GraphPad, La Jolla, CA). Differences were considered to be statistically significant at the P < 0.05 level. Data from one animal were excluded from statistical analysis because of a consistent lack of responsiveness, which correlated with an unusually high basal level of insulin (>150 μIU/ml). Of the nine animals treated with XMetA, two animals were found to be positive for antidrug antibody, one starting in the second week and the other in the sixth week of treatment. Data from these animals obtained at time points subsequent to the antidrug antibody generation (and concurrent loss of efficacy) were excluded from the statistical analysis. For the lower-dose acute test of 1.5 mg/kg, one animal was excluded from the analysis because of the lack of drug exposure confirmed by serum drug analysis.

Results

Reduction in Fasting Glucose 1 Day after s.c. Dosing of XMetA at 3 or 10 mg/kg in Diabetic Cynomolgus Monkeys. Acute efficacy of SC XMetA at 3 and 10 mg/kg (n = 4 or 5/group) was evaluated in a heterogeneous group of spontaneous type 2 diabetic cynomolgus monkeys that were not receiving exogenous insulin therapy. Monkeys selected for the study displayed overnight FSG levels of 146–270 mg/dL, thus validating their marked diabetic state (see Table 1). Furthermore, these monkeys showed elevated HbA1c levels (range 6.3–13.4%), and the insulin and C-peptide levels generally ranged from normal to moderately high with a mean value of 28 μIU/ml and 1.3 nmol/liter, respectively. One monkey had a particularly elevated basal insulin of 162 μIU/ml. The heterogeneity in metabolic parameters in this group of monkeys reflects the human disease and suggests this model may be particularly clinically relevant. The results indicate that a single s.c. administration of XMetA at 3 and 10 mg/kg substantially reduced fasting hyperglycemia in eight of the nine monkeys (from 237 ± 25 mg/dL to 184 ± 12 mg/dL in the 3 mg/kg group and from 211 ± 32 mg/dL to 123 ± 34 mg/dL in the 10 mg/kg group). The one monkey that did not show a decrease in fasting blood glucose was the animal exhibiting a high basal insulin level of 162 μIU/ml. The average reduction in fasting blood glucose in the responders was 53 (∼21%) and 88 mg/dL (∼45%) in the 3 and 10 mg/kg dose groups, respectively.

Repeated SC XMetA Treatment Results in Sustained Reduction in Fasting Glucose over a 6-Week Dosing Period in Diabetic Cynomolgus Monkeys. The safety and longer-term ability of XMetA to control hyperglycemia was evaluated via a dosing regimen of once weekly s.c. administration at 10 mg/kg for 6 weeks. The data indicate that XMetA treatment resulted in sustained weekly reductions in fasting hyperglycemia of approximately 30% on average throughout the study, whereas untreated animals from the same screening cohort showed some progression in FSG levels during the ~2-month study period (Fig. 2). The average glucose level of the treated animals after vehicle (week 1) was 264 ± 23 mg/dL, and the average glucose level of the untreated animals at screening (study start) was 146 ± 30 mg/dL. Nonfasting (postprandial) hyperglycemia (1 PM glucose levels) also tended to be reduced (Fig. 3). No significant changes were seen in the 7 PM glucose values over the course of the study (data not shown). Reductions in HbA1c (from a mean value of 10.5% after vehicle treatment to 9.3% after the last XMetA dose; absolute reduction of 1.2%) and C-peptide levels compared with the vehicle control baseline were observed over 6 weeks of treatment (Fig. 4). Thus, XMetA treatment improves glycemic control after acute or subchronic dosing regimens and may have β-cell/insulin-sparing actions in NHPs.

XMetA Is Efficacious in Reducing Hyperglycemia at a Dose as Low as 1.5 mg/kg. An evaluation of the magnitude and duration of glucose-lowering efficacy across doses of 1.5, 3, and 10 mg/kg is presented in Fig. 5, A–C. In Fig. 5, A and B, the daily AM blood glucose profile of the four to eight animals per group after 3 (Fig. 5A) or 10 (Fig. 5B) mg/kg dosing is compared with the response in the vehicle control dosing week. Nonfasting hyperglycemia was not significantly reduced (data not shown). In a follow-up study, even lower-dose efficacy (1.5 mg/kg) in some of the same monkeys after a washout period was evaluated (Fig. 5C). The combined data suggest that a single XMetA administration at doses between 1.5 and 10 mg/kg yielded similar magnitude reductions in morning hyperglycemia, and the duration of lowering was ~6 days at the high dose and ~4-5 days at 1.5 and 3 mg/kg. For all doses, the peak effect was 1 or 2 days after administration.
Additionally, in the 1.5 mg/kg test group, nonfasting hyperglycemia (Fig. 5C) and serum C-peptide levels (Fig. 5D) were significantly decreased for 4 to 5 days postdosing, whereas insulin levels were not substantially altered (data not shown). Together, the data show that a single SC administration of XMetA at 1.5 mg/kg was equally efficacious in reducing fasting hyperglycemia as 10 mg/kg, with a reduction of nearly 40% in FSG observed on the day after dosing for both dose levels (Fig. 6).

**XMetA Did Not Cause Hypoglycemia and Did Not Significantly Alter Body Weights and Food Consumption in Diabetic Cynomolgus Monkeys.** None of the animals became hypoglycemic (defined as having an FSG level <40 mg/dl or showing clinical symptoms) during the study, even at the highest dose level (10 mg/kg). In addition, no significant changes in body weights and food consumption from vehicle control pretreatment measurements were noted in the animals during the treatment period. Body weights and food intake were 5.52 ± 0.9 kg and 173 ± 22 g/24 hours, respectively, after vehicle treatment and 6.29 ± 1.1 kg and 173 ± 25 g/24 hours after the last XMetA dose. XMetA treatment was generally well tolerated, with no abnormal clinical signs or gastrointestinal tolerability issues and no injection-site reactions.

**Serum XMetA Concentration and Antidrug Antibody Assessment.** Serum XMetA levels were dose-incremental and averaged between approximately 3 to 18 μg/ml on day 2 (~20 hours postdose) across all dose levels. Based on the concentration-time profile obtained after a single s.c. administration of 1.5, 3, and 10 mg/kg, the absorption was slow and highly variable, and the time to reach maximal serum concentration (Tmax) was approximately day 2 at the lower two dose levels and day 3 at the 10 mg/kg dose; the terminal half-life was 3 to 5 days. Because of the differences in the rate of absorption, the mean serum drug concentration on day 2 was not dose-proportional; however, on day 5, it was dose-proportional. At the 10 mg/kg dose, the mean concentration on day 5 was comparable to the mean concentration on day 2 (7.4 vs. 5.6 μg/ml, respectively) and proportionally higher than at 1.5 and 3 mg/kg. Figure 7 shows a scattergram of the serum XMetA data on days 2 and 5 postdose and the corresponding fasting blood glucose response (expressed as percent change from day 1 values). We were best able to correlate serum XMetA concentration versus reduced hyperglycemia in the 1.5 and 10 mg/kg dose groups, and these are depicted in Fig. 7. The correlation coefficient was −0.25, and although not significant, there appears to be a trend for a greater reduction with increased XMetA concentration.

Results from the antidrug antibody assay revealed that two animals were positive for antidrug antibody, starting in the second and sixth week of XMetA treatment, respectively. The immunogenicity response was clearly evident by detectable serum XMetA levels precipitously dropping from approximately 15 μg/ml steady-state levels to <0.5 μg/ml over 2 to 5 days. Because XMetA is a fully human antibody, such a response is not uncommon or unexpected in monkeys, but the likelihood of an immunogenicity response in humans is expected to be low.

**Discussion**

We have established that XMetA, a fully human monoclonal antibody that is a high-affinity partial agonist of the IR, can ameliorate hyperglycemia in a stringent and clinically relevant NHP model of type 2 diabetes. XMetA has previously been characterized in vitro and has shown in vivo efficacy in diabetic mice. In particular, XMetA treatment normalized fasting hyperglycemia and reduced HbA1c levels in insulin-resistant and insulinopenic diabetic mice but with a reduced risk of hypoglycemia, oncogenicity, and weight gain (Bhaskar
et al., 2012; 2013). In the current study, the glucoregulatory activity of s.c. XMetA was evaluated in obese, spontaneously diabetic NHPs. The data indicate that XMetA reduced hyperglycemia after acute and subchronic dosing in cynomolgus monkeys. Efficacy was evident acutely at doses as low as 1.5 mg/kg, and efficacy was maintained on repeat administration for at least 6 weeks (at 10 mg/kg). Moreover, the multiweek dosing yielded a significant absolute reduction in HbA1c levels of 1.2% after 6 weeks of treatment. Together, the data support XMetA as a novel therapeutic approach for the management of T2DM.

Spontaneously obese/diabetic cynomolgus macaques provide an excellent animal model of type 2 (insulin-resistant) diabetes since these monkeys can develop metabolic conditions and display clinical features similar to those seen in humans, including obesity, hyperglycemia, severe insulin resistance, and increased HbA1c (Wagner et al., 2001; Bauer et al., 2011; Harwood et al., 2012). In this study, XMetA decreased both fasting and nonfasting glucose in diabetic monkeys across test doses, with reductions in fasting glucose ranging from 20%–40%. In this heterogeneous group of monkeys, XMetA proved to be effective across a broad range of basal hyperglycemia and insulinemia. One poor responder had an especially high basal level of insulinemia (160 mIU/ml), and we speculate that this animal was therefore particularly insulin resistant or had downregulated IRs. The glucose-lowering effects of XMetA compare favorably to those observed with other antidiabetic drugs in the same colony of diabetic cynomolgus monkeys: oral treatment with metformin at 25 mg/kg twice daily or with the peroxisome proliferator-activated receptor (PPAR)-γ agonist rosiglitazone at 1 mg/kg daily resulted in reductions of 10% and 21%, respectively, in fasting hyperglycemia over 2–4 weeks of treatment (Crown Bioscience, 2015). Other studies report that antidiabetic drugs, including rosiglitazone (Gee et al., 2004) and the PPAR-α agonist CP-900691 (Wagner et al., 2010), improved glycemic control in diabetic cynomolgus monkeys, but these evaluations were done in animals dependent on exogenous insulin therapy.

The reduced hyperglycemia (and HbA1c) after XMetA treatment occurred in tandem with reduced serum C-peptide levels. Fig. 5. (A, B) XMetA reduced hyperglycemia over 5 to 6 days after a single s.c. administration at 3 mg/kg (A) and 10 mg/kg (B) in diabetic cynomolgus monkeys compared with vehicle administration. (C, D) XMetA reduced fasting and nonfasting hyperglycemia (C) and C-peptide levels (D) over 5 days after a single s.c. administration at 1.5 mg/kg in diabetic cynomolgus monkeys. Days 2 and 5 were scheduled fasting days; but, since monkeys are not nocturnal and generally do not eat at night, the 7 AM glucose measurements on the other days were considered “fasting” levels for the purpose of these graphs. Values represent mean ± S.E.M. (A, n = 4; B, n = 8; C and D, n = 3). *p < 0.05; **p < 0.01 versus vehicle (A, B, D; repeated measures two-way ANOVA) or versus day 1 (C; repeated-measures ANOVA using a linear mixed-effects model and test-control contrast matrix).

**Fig. 6.** XMetA reduced FSG 1 day after a single s.c. administration at 1.5 and 10 mg/kg in diabetic cynomolgus monkeys. The average glucose baseline values were 270 ± 61 mg/dl and 249 ± 35 mg/dl for the 1.5 and 10 mg/kg groups, respectively. Values represent mean ± S.E.M. (n = 3 or 4). **P < 0.01 versus baseline (BL) (one-way ANOVA).**

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**Fig. 7.** Scattergram of the serum XMetA data and the corresponding percent change in fasting blood glucose. Percent change in fasting blood glucose was the day 2 or day 5 postdose 7 AM glucose values compared with day 1/predose data for animals in the 1.5 and 10 mg/kg dose groups. The correlation coefficient was −0.25 (nonparametric Spearman correlation test, not significant).
levels, potentially supporting an insulin/β-cell sparing mechanism. Reductions in both hyperglycemia and C-peptide levels are consistent with the previous studies in insulinopenic diabetic mice (Bhaskar et al., 2012) and in hyperinsulinemic DIO mice after 4 weeks of dosing (Bhaskar et al., 2013). HbA1c levels reflect long-term exposure to elevated glucose levels (i.e., functions as a measure of glycemic control in the previous 8–12 weeks in these monkeys), considering that the lifespan of a macaque erythrocyte is 60–85 days (Moore, 2010). HbA1c levels may conceivably be further reduced with prolonged treatment, with a projected reduction approximating 2% after 2 months of treatment in diabetic cynomolgus monkeys based on the observed trendline. Since the XMetA antibody, as a fully human antibody, is anticipated to have a longer half-life in humans (typically greater than 1 week) (Lonberg, 2008) and hence may yield more sustained glucose lowering during the week, we speculate that XMetA has even greater potential in reducing HbA1c in diabetic humans. Moreover, as 1.5 mg/kg appeared as effective as any other dose for at least several days postadministration, a human equivalent dose projection would translate to ~1 mg/kg and with reduced hyperglycemia throughout a weekly dosing regimen.

The serum drug analysis data show that serum XMetA concentrations were generally dose-proportional and sustained week to week. Efficacy of XMetA was correlated with the presence of XMetA in the serum at mean concentrations ranging from 3–18 μg/ml. In general, higher concentrations were observed on day 5 at the 10 mg/kg dose, thereby correlating with the longer-lasting efficacy versus the lower two dose levels. Indeed, an apparent pharmacokinetic-pharmacodynamic relationship was evident from the poor glucose-lowering response in animals with no or low drug exposure. Moreover, in two animals, loss of efficacy in reducing hyperglycemia coincided precisely with the development of an antidrug antibody response. Generation of an antidrug antibody response would be at a much lower level, if at all, in human patients, as XMetA is a fully human antibody.

The present study also demonstrates that XMetA was safe and well-tolerated in NHPs with no evidence of local irritation or gastrointestinal tolerability issues and no weight gain. Importantly, no indication of hypoglycemia was observed at all doses tested, even at 10 mg/kg, consistent with previous reports of XMetA studies in mice (Bhaskar et al., 2012; 2013) and in insulin-dependent spontaneous type 2 diabetic rhesus monkeys (Zhao et al., 2014). This may possibly be explained by the fact that XMetA is only a partial agonist of the IR. Moreover, the combined hyperglycemic and insulin-resistant status of the diabetic subjects in this study may favor glucose lowering to a more normal range versus hypoglycemia. Taken together, this suggests XMetA has an improved therapeutic index compared with full IR agonists. Indeed, a preliminary therapeutic index of >6.7-fold apparent in the study herein would imply greater dosing flexibility than is feasible with insulin.

The tendency toward weight gain with insulin and PPAR activators is one of the potential barriers for their use in patients with T2DM, especially in obese patients. Weight gain from insulin use is related to its effect to increase body fat and lean mass through reductions in glycosuria, its anabolic effects on adipose tissue, as well as its appetite-enhancing effects (Barnett et al., 2007). One study demonstrated that each 1% absolute reduction in HbA1c achieved with neutral protamine Hagedorn (NPH) insulin was associated with a 2-kg increase in body weight in patients with T2DM (Makimattila et al., 1999). In addition, increasing insulin availability (or dosage) to overcome the blockade of the metabolic pathway in insulin resistance in obesity and type 2 diabetes has been reported to lead to an exaggerated activation of the mitogenic pathway. The potential long-term clinical consequences of this exaggerated mitogenic activity may be related in part to the side effects associated with chronic intensive insulin therapy in patients with type 2 diabetes (Lebovitz, 2011). As XMetA is a partial agonist that predominantly activates the metabolic pathway with little or no mitogenic activation, the weight gain seen with insulin or PPAR agonists is unlikely following XMetA treatment.

The safety profile of XMetA (a low risk of hypoglycemia, weight gain, and gastrointestinal side effects) therefore differentiates it from current glucose-lowering medications, including insulin, metformin, sulfonylurea, thiazolidinediones (pioglitazone and rosiglitazone), and glucagon-like peptide 1 agonists (Nathan et al., 2009; American Diabetes Association, 2015). Moreover, the duration of effect (~1 week after a single administration) and sustained effect with weekly repeated administration—with no tachyphylaxis—supports the potential for once weekly dosing in the clinic, in contrast to, for example, long-acting basal insulins, glucagon-like peptide 1 agonists, and α-glucosidase inhibitors, which require daily to once weekly injections. Furthermore, XMetA can act in concert with insulin and other T2DM drugs. Target tissues for the observed XMetA efficacy in diabetic monkeys were not determined; however, the reduction in both fasting and nonfasting (postprandial) hyperglycemia suggests some involvement of liver and muscle in the glucose modulation (Bedinger et al., 2015b).

Taken together, the data indicate that XMetA improved glycemic control (and in some animals even normalized hyperglycemia) in a monkey T2DM model without causing hypoglycemia or promoting weight gain. Its efficacy, tolerability, and attractive therapeutic index support further development of XMetA as a novel, first-in-class, and potentially improved pharmacotherapy with broad utility in type 2 diabetes. XMetA may be additionally useful in hyperglycemic conditions wherein insulin activation of receptors is defective (Semple et al., 2011), but allosteric agonism may compensate.

Acknowledgments

The authors thank Dr. Annemarie Ledeboer for assisting with data and statistical analyses and manuscript preparation and Drs. Ira Goldfine and Dan Bedinger for valuable comments.

Authorship Contributions

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Antidiabetic Activity of a Long-Acting IR Partial Agonist


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