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IgG-Enzyme Fusion Protein: Pharmacokinetics and Anti-Drug Antibody Response in Rhesus Monkeys

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Abstract

The chronic administration of recombinant fusion proteins in preclinical animal models may generate an immune response and the formation of anti-drug antibodies (ADA). Such ADAs could alter the plasma pharmacokinetics of the fusion protein, and mask any underlying toxicity of the recombinant fusion protein. In the present study, a model IgG-enzyme fusion protein was evaluated with chronic dosing of Rhesus monkeys. The IgG domain of the fusion protein is a genetically engineered monoclonal antibody (MAb) against the human insulin receptor (HIR), which is shown to cross-react with the primate insulin receptor. The enzyme domain of the fusion protein is human iduronidase (IDUA), the lysosomal enzyme mutated in Mucopolysaccharidosis Type I (MPSI). MPSI affects the brain, but enzyme replacement therapy is not effective for the brain, because IDUA does not cross the blood-brain barrier (BBB). The HIRMAb domain of the fusion protein acts as a molecular Trojan horse to deliver the IDUA across the BBB. The HIRMAb-IDUA fusion protein was administered to Rhesus monkeys with weekly intravenous infusions of 3–30 mg/kg for 6 months, and the pharmacokinetics, immune response, and tissue toxicology was assessed. The pharmacokinetics of plasma clearance of the fusion protein was determined with measurements of plasma IDUA enzyme activity. ADAs formed during the course of the 6 months of treatment, as determined by a sandwich ELISA. However, the plasma clearance of the fusion protein at the start and end of the 6-month study was comparable at all drug doses. Fusion protein administration for 6 months showed no evidence of chronic tissue toxicity. These studies demonstrate that the immune response produced with chronic treatment of primates with an IgG-enzyme fusion protein has no effect on the pharmacokinetics of plasma clearance of the fusion protein.

INTRODUCTION

Drug development of recombinant proteins for the brain is difficult, since these large molecule drugs do not cross the blood-brain barrier (BBB). One approach to the BBB problem is the re-engineering of the protein drug as an IgG fusion protein. The IgG domain is a monoclonal antibody (MAb) directed against an endogenous receptor transporter at the BBB, such as the insulin receptor or transferrin receptor.[1] The MAb domain of the fusion protein acts as a molecular Trojan horse to ferry the fused therapeutic domain across the BBB. An important consideration in the drug development of BBB-penetrating IgG fusion proteins is the immune response following long-term treatment. The formation of anti-drug antibodies (ADA) could alter the fusion protein clearance from blood and mask any underlying toxicity of the IgG fusion protein.[2] These issues were addressed in the present

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study, which measures the plasma pharmacokinetics (PK) of an IgG-enzyme fusion protein at the start and at the end of 6 months of chronic, weekly intravenous (IV) infusions in Rhesus monkeys. The ADA titer was measured at monthly intervals during the course of 6 months of treatment, and a tissue toxicologic evaluation was performed on all primates at the end of the study.

The IgG fusion protein tested in this study is an IgG-lysosomal enzyme fusion protein.[3] The IgG domain of the fusion protein is a genetically engineered MAb against the human insulin receptor (HIR), designated the HIRMAb. This domain binds the endogenous insulin receptor on the human BBB, and cross reacts with the insulin receptor in Old World primates such as the Rhesus monkey.[4] The enzyme domain of the fusion protein is human α-L-iduronidase (IDUA), and the fusion protein is designated the HIRMAb-IDUA fusion protein.[3] IDUA is a lysosomal enzyme which is mutated in Mucopolysaccharidosis (MPS) Type I, or MPSI.[5] MPSI can affect the brain, a condition called Hurler's syndrome. MPSI is treated with enzyme replacement therapy (ERT) using recombinant IDUA.[6] However, ERT does not treat the brain in Hurler's syndrome,[7] because the large molecule IDUA enzyme does not cross the BBB.[8] To enable BBB penetration, the IDUA enzyme has been re-engineered as an IgG-IDUA fusion protein. Chronic twice-weekly IV injections of Hurler mice with 1 mg/kg IgG-IDUA fusion protein for 8 weeks results in a reduction in lysosomal storage bodies in the brain, as well as a reduction in glycosoaminoglycans in peripheral tissues.[9]

The plasma PK profile of the HIRMAb-IDUA fusion protein in Rhesus monkeys was evaluated with measurements of the plasma IDUA enzyme activity. The use of plasma IDUA enzyme activity as a measure of the fusion protein concentration in plasma was validated with an ELISA. The sandwich ELISA measured the concentration of the HIRMAb-IDUA fusion protein, based on capture and detector reagents that bound to both the HIRMAb and the IDUA domains of the fusion protein. A separate ELISA was developed to measure the ADA response against the fusion protein. Over the course of 6 months of treatment, the ADA titer increased in plasma, and some monkeys developed hypersensitivity reactions to fusion protein infusion. The effect of the immune response on plasma clearance of the HIRMAb-IDUA fusion protein was evaluated by comparison of the pharmacokinetics of fusion protein clearance from plasma at the start (week 1) and end (week 25) of the 6 months of treatment. The study shows that the ADAs formed against the IgG-enzyme fusion protein do not alter the plasma bioavailability of the fusion protein, and thus do not mask any underlying toxicity of chronic fusion protein administration.

EXPERIMENTAL PROCEDURES

Production and analysis of HIRMAb-IDUA fusion protein

A high producing, stably transfected Chinese hamster ovary (CHO) line producing the HIRMAb-IDUA fusion protein was generated as described previously.[10] The CHO cells were cultivated in a 50L bioreactor under perfusion-mode conditions in serum free medium. The HIRMAb-IDUA fusion protein, also designated AGT-181,[10] was purified by protein A affinity chromatography, cation exchange chromatography, anion exchange chromatography, and nano-filtration, and 17 bio-analytical tests showed the drug product passed all specifications with respect to purity, identity, potency, quality, and safety. Stability studies showed the HIRMAb-IDUA fusion protein was stable as a sterile liquid stored at 4°C for up to 2 years. Over 60 grams of HIRMAb-IDUA fusion protein was manufactured for this study. The HIRMAb-IDUA fusion protein is a hetero-tetrameric molecule comprised of 2 heavy chains (HC) and 2 light chains (LC) formed by fusion of the mature human IDUA to the heavy chain (HC) of the genetically engineered chimeric HIRMAb.[3,10] The 627-amino acid (AA) human IDUA is fused to the carboxyl terminus of the HC via a 2-AA (Ser-Ser) linker. The HIRMAb HC is comprised of a 113-AA variable

(V)-region, and a 330-AA human IgG1 constant (C)-region. The LC is comprised of a 108-AA V-region, and a 106-AA human kappa C-region. The HIRMAb-IDUA fusion protein is a bi-functional molecule that both expresses high IDUA enzyme activity and binds the HIR with high affinity characterized by a binding constant <1 nM.[3,10]

Study design

Rhesus monkeys (*Macaca mulatta*) of mixed sex were used for all studies, and were housed at MPI Research, Inc. (Mattawan, MI) in stainless steel cages in a controlled environment (18 to 28° C and 30–70% relative humidity) on a 12-h light/dark cycle. Lab Diet Certified Primate Diet (PMI Nutrition International) was provided twice daily. Tap water was provided ab libitum. All aspects of the primate study performed at MPI Research was conducted in strict compliance with the United States Food and Drug Administration Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58. All procedures were in compliance with the *Animal Welfare Act Regulations*, and were approved by the Institutional Animal Care and Use Committee.

Juvenile Rhesus monkeys (20 females, 20 males), with starting body weight of 2.6–5.0 kg (mean 3.5 kg) were divided into 4 treatment groups: (a) 12 monkeys (6 females, 6 males) were treated with vehicle; (b) 8 monkeys (4 females, 4 males) were treated with low dose drug (3.0 mg/kg); (c) 8 monkeys (4 females, 4 males) were treated with mid dose drug (9.0 mg/kg); (d) 12 monkeys (6 females, 6 males) were treated with high dose drug (30.0 mg/kg). Drug vehicle is 0.01 M sodium acetate/0.14 M NaCl/0.001% Tween 80/pH=5.5 (ABST). Drug was infused over 30 minutes in 50 mL of normal saline with or without 10% dextrose, and was administered weekly for 26 consecutive weeks. Animals were euthanized 24 hours after the last dose at week 26, except for 4 monkeys (2 females, 2 males) in the vehicle group and 4 monkeys in the high dose group (2 females, 2 males), which constituted a recovery group, and were euthanized at 30 weeks, or 4 weeks following the last drug dosing. Clinical findings, food consumption, and body weight were recorded weekly. Electrocardiogram and ophthalmoscopic exams were performed at the start and end of the study. Clinical chemistry was measured on plasma obtained at the start of the study, at 13 weeks, and at termination, and included 9 hematologic tests, 2 coagulation tests, 16 metabolic tests, and 10 urinalysis tests.

The brain (cerebrum, cerebellum, spinal cord) and 33 peripheral organs were blocked and fixed in formalin, and examined with hematoxylin and eosin by a certified pathologist at MPI Research following euthanasia at 26 or 30 weeks. Bone marrow, large intestine, small intestine, spinal cord, and pancreas were all tested at 3 locations of the organ. The brain blocks were prepared according to Garman.[11] The brain was also examined for astrogliosis with glial fibrillary acidic protein (GFAP) immunohistochemistry, and for neurodegeneration with fluoro Jade B fluorescence microscopy.

Blood was removed for PK analysis at multiple time points (0, 2, 5, 30, 35, 90, 180, 360, and 1380 min) following the start of the 30 min drug infusion. PK studies were performed both at the start (week 1) and end (week 25) of the study. Plasma samples removed at the start of the study were analyzed with both the HIRMAb-IDUA ELISA and the IDUA enzyme activity. The HIRMAb-IDUA ELISA could not be used on plasma samples removed at the end of the study, owing to interference by anti-drug antibodies (ADA) in primate plasma. Therefore, the PK analysis at the end of the study was performed with the plasma IDUA enzyme activity measurements. Blood was removed for plasma collection at monthly intervals for immune response ELISA and measurement of ADA.

Some animals in the mid dose (9 mg/kg) and high dose (30 mg/kg) treatment groups showed clinical signs of hypersensitivity (facial flushing, shortness of breath) by the third week of

dosing. Therefore, diphenhydramine (DPH), 1–2 mg/kg intra-muscular, was administered prophylactically to all monkeys in the mid dose and high dose groups prior to drug infusion for a 5-week period during weeks 3 through 7. Pre-treatment with DPH was discontinued for drug dosing during weeks 8 through 26. During this period, some animals demonstrated post-infusion hypersensitivity and DPH was administered to these animals for treatment of specific symptoms. Symptoms generally resolved within 10–15 min after DPH treatment. No animals in the vehicle or low dose (3 mg/kg) received any DPH during the course of the 6-month study.

Immunoreactive HIRMAb-IDUA fusion protein measurement by ELISA

The plasma concentration of the HIRMAb-IDUA fusion protein was determined with a sandwich ELISA described previously.[10] The capture reagent is a complex of the lectin-purified recombinant HIR extracellular domain (ECD) and a murine MAb against the HIR ECD. The detector reagent is a complex of a rabbit polyclonal antiserum against human recombinant IDUA and a conjugate of alkaline phosphatase and a goat anti-rabbit IgG secondary antibody. Following the addition of the 4-nitrophenylphosphate substrate, absorbance (Abs) at 405 nm was measured. A standard curve of 0–1000 ng/mL HIRMAb-IDUA fusion protein was included in each assay. The following equation: $Abs = (Amax \cdot S)/(EC50 + S)$, was fit to the ELISA data, where Amax is the maximal absorbance, EC50 is the concentration of HIRMAb-IDUA fusion protein that yields 50% of maximal absorbance, and S is the concentration of HIRMAb-IDUA fusion protein. In a typical assay, the Amax =1.40 ± 0.03 and EC50=53.0 ± 4.7 ng/mL (R2=0.991). Both domains of the fusion protein, the HIRMAb domain and the IDUA domain, must be intact in order for the fusion protein to register a signal in the ELISA. Standard curves were analyzed by non-linear regression analysis using the PAR subroutine of the BMDP2007 Statistical Software (Statistical Solutions, Dublin, Ireland).

IDUA enzyme activity

IDUA enzyme activity was measured with a fluorometric enzymatic assay described previously,[3] which uses as substrate 4-methylumbelliferyl α-L-iduronide (Glycosynth, Ltd., Cheshire, England). Enzyme activity was measured over 60 min at 37°C, and reported as units/mL, where 1 unit = 1 nmol/hr, and 1 kilounit = 1000 nmol/hr. The IDUA specific activity of the HIRMAb-IDUA fusion protein was 796 units/ug protein.

Pharmacokinetics

The plasma concentration, A(t), or the plasma IDUA enzyme activity, was divided by the injected dose (ID) and converted to % ID/mL. A mono-exponential function, $A(t) = A \cdot e^{-kt}$, where A is the maximal %ID/mL, k is the mono-exponential rate constant, and t = the time after completion of the 30 min drug infusion (5 to 360 min), was fitted to the plasma concentration data. The plasma concentration at 1380 min after drug administration was not included in the PK analysis, because the fusion protein was no longer detectable in plasma at this time point. The maximal fusion protein concentration in plasma following the 30 min infusion, the C_{max}, was determined directly with either the ELISA or the IDUA enzyme activity. The pharmacokinetic parameters were determined from the following relationships:

half-time of clearance (T1/2) = ln 2/k

volume of distribution (V_{ss}) = 100/A

area under the concentration curve (AUC) = A/k

clearance (CL) = 100/(A/k)

The PK data fits and parameter estimates were made by non-linear regression using the PAR subroutine of the BMDP2007 Statistical Software.

Biotinylation of the HIRMAb-IDUA fusion protein

Biotinylated HIRMAb-IDUA fusion protein was used in both the immune response sandwich ELISA and in the Tissue Cross-Reactivity (TCR) study. The HIRMAb-IDUA fusion protein, or the human IgG1k isotype control, was biotinylated with sulfo biotin-LC-LC-N-hydroxysuccinimide, where LC=long chain (#21338, Pierce Chemical Co., Rockford, IL) as described previously.[10] The biotinylation of the IgG was confirmed by SDS-PAGE and Western blotting, where the blot was probed with a mixture of avidin and biotinylated peroxidase. The control HIRMAb-IDUA fusion protein, or the control human IgG1, gave no reaction in the Western blot, whereas the biotinylated protein was strongly visualized at the appropriate molecular size for both heavy chain and light chain. The effect of biotinylation on the HIRMAb-IDUA fusion protein binding to the HIR ECD was measured by ELISA, as described previously.[3] The biotinylated HIRMAb-IDUA fusion protein bound with high affinity to the HIR ECD with an ED50 of 0.34 ± 0.03 nM. The effect of biotinylation on the IDUA enzyme activity of the HIRMAb-IDUA fusion protein was measured with a fluorometric enzymatic assay, which showed the IDUA enzyme activity of the biotinylated HIRMAb-IDUA fusion protein was unchanged from the IDUA enzyme activity of the non-biotinylated HIRMAb-IDUA fusion protein.

Immune response ELISA

Plasma was obtained monthly for measurement of ADA titers and 7 days after the most recent infusion of HIRMAb-IDUA fusion protein, when plasma concentrations of the fusion protein were undetectable. The level of ADA against the HIRMAb-IDUA fusion protein was measured with a bridging ELISA in 96-well plates as described previously.[10] The capture reagent is the HIRMAb-IDUA fusion protein (250 ng/well) and the detection reagent is a complex of biotinylated HIRMAb-IDUA fusion protein (25 ng/well) and a conjugate of streptavidin and horseradish peroxidase (500 ng/well). Owing to the bivalency of ADA binding, the HIRMAb-IDUA fusion protein serves as both the capture and detector reagent. Each well contained 100 uL monkey plasma diluted in PBS. Following addition of the o-phenylenediamine substrate, absorbance (Abs) was measured at 490 nm and 655 nm, and (A490-A655) was computed. The Abs at 655 nm accounts for light-scattering and is a small fraction of the A490. Plasma from the treated monkeys was removed at 0, 4, 8, 13, 16, 20, and 24 weeks of the study, and the (A490-A655) was measured for each animal at each time point using dilutions of monkey plasma prepared in 0.01 M Na_2HPO_4/0.15 M NaCl/pH=7.4 (PBS). For the time course of ADA formation over 24 weeks, plasma from individual monkeys was diluted 1:50 in PBS. For the dilution curve of ADA formation, an aliquot of plasma from each monkey (10 uL) in each dosing group, all at week 20 of the study, was pooled and the pool was diluted 1:50, 1:100, 1:300, 1:1000, 1:3000, and 1:10,000 in PBS. These dilutions were tested against one of 3 different capture reagents: (a) the HIRMAb-IDUA fusion protein, (b) the HIRMAb alone without the enzyme, and (c) the isotype IgG control, human IgG1κ. The ADA titer was expressed as optical density (OD) per uL plasma, and was determined from the (A490-A655), multiplied by the plasma dilution, and divided by the volume (uL) of plasma per well.[12] The positive control in the ADA ELISA was a rabbit antiserum prepared against the HIRMAb-IDUA fusion protein (Prosci, Inc., Poway, CA).

Tissue cross-reactivity

A Tissue-Cross Reactivity (TCR) study of the HIRMAb-IDUA fusion protein was performed with autopsy, archival tissues from 3 healthy humans and 2 healthy Rhesus monkeys using the avidin-biotin immunoperoxidase method under GLP conditions by

Charles River Laboratories Pathology Associates (Frederick, MD). Fresh tissue was frozen in cyromolds in Optimal Cutting Temperature compound, and frozen sections (5 micron) were prepared with a cryostat. A total of 35 human and 34 Rhesus monkey organs were examined, as described previously.[4] The primary antibody was either the biotinylated HIRMAb-IDUA fusion protein, or biotinylated human IgG1κ, used at concentrations of 2 and 15 ug/mL. Positive tissue staining was verified with an anti-α_2-microglobulin antibody. Specificity for the HIR was examined with spot slides using recombinant HIR (R&D Systems) as a positive control, and human parathyroid hormone-related protein (PTHrP)-1–34 (Sigma Chemical Co.) as a negative control.

Statistical analysis

Statistical significance was determined at the $p<0.05$ level using the analysis of variance (ANOVA) with Bonferroni correction, or Student's t-test.

Results

The Tissue Cross Reactivity (TCR) study showed comparable binding of the HIRMAb-IDUA fusion protein to human and Rhesus monkey tissues at both the 2 and 15 ug/mL concentrations of the biotinylated fusion protein. Biotinylated human IgG1k yielded no tissue reactivity at either concentration. Biotinylated HIRMAb-IDUA gave strong staining on spot slides of HIR, but did not react with spot slides of human PTHrP. There was detectable immune staining in virtually all human and primate tissues. In brain, the HIRMAb-IDUA fusion protein bound to both the neuropil and the endothelium in the cerebrum, cerebellum, and spinal cord. The TCR study supports the use of the Rhesus monkey as a test species in which the HIRMAb-IDUA fusion protein is biologically active.

The plasma concentration profile of the immunoreactive HIRMAb-IDUA fusion protein at the start of the study is shown in Figure 1 for the low dose (3 mg/kg), mid dose (9 mg/kg), and high dose (30 mg/kg) treatment groups. A compartmental model of plasma clearance of the fusion protein was fit to the plasma concentration data to produce the PK parameters listed in Table 1. The C_{max} and plasma AUC were directly proportional to the injection dose (ID), whereas the V_{ss}, and to a lesser extent, the systemic clearance (CL), were inversely related to the ID (Table 1).

The plasma IDUA enzyme activity profile is plotted in Figure 2 following IV injection of the HIRMAb-IDUA fusion protein at the start (week 1) of the study. A compartmental model was fitted to these data of plasma clearance of the fusion protein to produce the PK parameters listed in Table 2 (top). The PK parameters determined with the plasma IDUA enzyme activity are comparable to the PK parameters determined with the plasma immunoreactive HIRMAb-IDUA fusion protein measurements, and the linear relationship between the plasma AUC at the 3 injection doses is shown in Figure 3.

The plasma IDUA enzyme activity profile is plotted in Figure 2 following IV injection of the HIRMAb-IDUA fusion protein at the end (week 25) of the study. A compartmental model of plasma clearance of the fusion protein was fitted to produce the PK parameters listed in Table 2 (bottom). The PK parameters of fusion protein clearance are comparable at the start and end of the 6 months of treatment (Table 2).

The immune response formed against the HIRMAb-IDUA fusion protein over the course of 24 weeks of weekly treatment was assessed with a sandwich ELISA (Methods) and 1:50 dilutions of individual primate plasma. The titer of anti drug antibody (ADA) increased after 4 weeks of treatment and reached a maximum by 12–15 weeks of treatment, with a general inverse relationship between ADA titer and injection dose (Figure 4). No immune response

was detected in the vehicle-treated monkeys (Figure 4). Plasma obtained at 20 weeks for all monkeys in each treatment group were pooled and diluted from 1:50 to 1:10,000, and the titer of ADA was measured with one of 3 capture reagents, including the HIRMAb-IDUA fusion protein (Figure 5A), the HIRMAb alone (Figure 5B), and the human IgG1k isotype control (Figure 5C). The titer, or OD/uL plasma, of ADAs are shown in Table 3. The average titer at all 3 doses of fusion protein was reduced 40% when the capture reagent was changed from the HIRMAb-IDUA fusion protein to the HIRMAb alone, and was reduced 91% when the capture reagent was changed from the HIRMAb-IDUA fusion protein to the IgG1k isotype control antibody (Table 3).

The formation of ADAs against the HIRMAb-IDUA fusion protein required treatment of hypersensitivity reactions with DPH (Methods), which was administered prophylactically to all monkeys in the mid dose and high dose groups between weeks 3 to 7 of the study. Between weeks 8 to 26, DPH was administered only for treatment of hypersensitivity reactions in specific monkeys. Between weeks 8 to 26 of the study, the number of monkeys requiring DPH treatment of hypersensitivity reactions following drug infusion was 0, 7.2%, and 13.5% in the low dose, mid dose, and high dose treatment groups, respectively. In the mid dose treatment group, 2 monkeys (1 male, 1 female) develop anaphylaxis at the last injection at week 26 and were euthanized for moribund condition.

No evidence of chronic toxicity was observed in any monkey during the 6 month treatment study. There were no significant changes in physical exam, food intake, EKG, ophthalmoscopic exam, body weights, or organ weights in any of the treatment groups relative to controls, including monkeys euthanized after a 1 month recovery period. There were no changes in 9 hematologic tests in any treatment group, except for a 1.6–1.8-fold increase in the lymphocyte count in the high dose group in males at weeks 13 and 26, which was not observed in females. There was no change in the prothrombin time or activated partial thromboplastin time coagulation profile in any group. There were no changes in 16 liver, renal, and metabolic function tests in any treatment group, except for a 1.5–2.3-fold increase in the plasma aspartate aminotransferase (AST) level in the high dose group in females at weeks 13 and 26, which was not observed in males. There was no change in 10 tests of the terminal urinalysis in any of the treatment groups, and glucosuria was not observed. Histological examination was made on brain and 33 peripheral tissues in all monkeys at the end of the study with hematoxylin and eosin staining. All brain sections were examined for neurodegeneration with fluoro Jade-B fluorescence microscopy and for astrogliosis with GFAP immunocytochemistry. No differences in the fluoro Jade B or GFAP staining were observed between the control and treated monkeys. There were no drug related microscopic findings in terminal or recovery animals in brain or 33 peripheral organs examined. The few microscopic findings were either common background findings in monkeys, or also observed in vehicle-treated monkeys and were considered unrelated to drug treatment.

Discussion

The results of this study are consistent with the following conclusions. First, the immunoreactive HIRMAb-IDUA fusion protein is rapidly cleared from plasma following IV infusion (Figure 1) with a systemic clearance of 4–8 mL/kg/min (Table 1). Second, the IV infusion of the HIRMAb-IDUA fusion protein results in a dose-dependent increase in plasma IDUA enzyme activity (Figure 2). Third, the clearance of the fusion protein from plasma is characterized by a linear pharmacokinetics profile, and the AUC of either the plasma immunoreactive fusion protein, or the plasma IDUA enzyme activity, is proportional to the dose of fusion protein (Figure 3). Fourth, chronic dosing over 26 weeks in Rhesus monkeys causes a time-dependent increase in anti-drug antibody (ADA) in plasma (Figure

4), and the ADA is directed against the V-region of the HIRMAb, the IDUA domain, and the antibody C-region of the HIRMAb-IDUA fusion protein (Table 3). Fifth, the ADA does not alter the PK of plasma clearance of the fusion protein, as the plasma profile of IDUA enzyme activity (Figure 2), and the PK parameters of plasma clearance (Table 2), are comparable at the start (week 1) and end (week 25) of the 6-month dosing study. Sixth, the HIRMAb-IDUA fusion protein has a favorable safety profile, and no evidence of chronic toxicity was observed following 6-months of weekly IV infusions of 3–30 mg/kg (Results).

The rapid clearance of the HIRMAb-IDUA fusion protein from plasma is comparable to the rapid systemic uptake of the IDUA enzyme alone, which is >90% cleared from plasma in the dog within 60 minutes of IV administration.[13] Whereas the tissue uptake of IDUA is mediated via the mannose 6-phosphate receptor (M6PR), the uptake of the HIRMAb-IDUA fusion protein by Hurler fibroblasts is independent of the M6PR, and is mediated by the insulin receptor.[3] The HIRMAb-IDUA fusion protein contains an intact human IgG1 C-region, which binds to Fc receptors (FcR), including the neonatal FcR. However, the neonatal FcR plays a reduced role in IgG clearance from blood in primates.[14] Following tissue uptake, the IDUA domain of the HIRMAb-IDUA fusion protein is triaged to the lysosomal compartment of target cells.[3] The IDUA is fused to the heavy chain of the HIRMAb via a short Ser-Ser linker, which is a sequence not targeted by common endopeptidases. Both the IDUA domain and the HIRMAb domain must remain intact in order to be detected by the sandwich ELISA (Methods). The identical plasma profile of the immunoreactive fusion protein (Figure 1) and the IDUA enzyme activity (Figure 2) is evidence that the fusion protein remains intact in blood prior to uptake by tissues. This stability of the fusion protein was verified in a prior study with Western blot analysis of plasma obtained from Rhesus monkeys injected with HIRMAb-IDUA fusion protein.[10]

Recombinant proteins are immunogenic in primates, and the immune response in primates is not predictive of the immune response in humans.[15–17] Chronic treatment of Rhesus monkeys with the HIRMAb-IDUA fusion protein results in the formation of ADAs in plasma (Figure 4). The ADAs are primarily directed against the HIRMAb V-region and the IDUA, and to a lesser extent the HIRMAb C-region (Figure 5, Table 3). The ADAs formed against the HIRMAb-IDUA fusion protein do not alter the plasma clearance of the fusion protein. This is demonstrated by the comparable rate of clearance of plasma IDUA enzyme activity at the start (week 1) and end (week 25) of the study (Figure 2, Table 2). The ADAs formed in Rhesus monkeys against the HIRMAb-IDUA fusion protein are not neutralizing antibodies (NAb). A NAb against the IDUA domain of the fusion protein would cause a reduction in the measured enzyme activity at the end of the study, and this was not observed (Figure 2). A NAb against the V-region of the HIRMAb domain would cause a reduction in plasma clearance of the fusion protein at the end of the study, and this was not observed (Figure 2, Table 2). Nevertheless, the ADAs formed against the HIRMAb-IDUA fusion protein caused hypersensitivity reactions during the last week of the 26-week study (Results), and such adverse reactions are reported in chronic dosing of primates with human recombinant proteins.[15,18] Immune responses against recombinant proteins are known to be more severe in primates,[15] and this was observed in the case of IgG-IDUA fusion proteins. In the present study, the HIRMAb-IDUA fusion protein, which is 83% human AA sequence and 17% mouse AA sequence, was administered chronically to Rhesus monkeys, and the maximal ADA titer formed against the IgG-IDUA fusion protein was 10 (Table 3). In contrast, a mouse-specific IgG-IDUA fusion protein, which was 84% mouse AA sequence and 16% rat AA sequence, was administered chronically by IV injections to mice, and the maximal ADA titer was <1.[9] Therefore, the immune response against a fusion protein with a human AA sequence in Rhesus monkeys is more than 10-fold greater than the immune response against a comparable fusion protein with a mouse AA sequence in mice. These

findings support conclusions from other studies that the immune response against human recombinant proteins in primates is not predictive of the immune response in humans.[15]

The chronic weekly IV infusions of the HIRMAb-IDUA fusion protein had no effect on hepatic, renal, or metabolic control during the 6-month study, with the exception of transient hypoglycemia induced by intravenous infusion of the high dose (30 mg/kg) of the fusion protein in normal saline.[19] The hypoglycemia at high doses is assumed to be caused by the HIRMAb domain of the HIRMAb-IDUA fusion protein, since hypoglycemia is not associated with chronic IDUA enzyme replacement therapy.[20] The nadir of hypoglycemia is 39 ± 5 mg% at 3 hours after IV infusion of the high dose of fusion protein, although severe hypoglycemia of 11–20 mg% was observed in 2 monkeys.[19] The hypoglycemia is observed only at the high dose (30 mg/kg) of HIRMAb-IDUA fusion protein, and is eliminated by adding dextrose to the saline infusion solution.[19] An intravenous glucose tolerance test performed at the end of the study showed there is no change in glucose tolerance following chronic treatment of Rhesus monkeys with any dose of the fusion protein.[19]

Chronic treatment of Rhesus monkeys for 6 months with the HIRMAb-IDUA fusion protein has no effect on any clinical or pathologic parameter measured. The results of this study do not confirm a study that reports 2–5 mm lesions in the pancreas of some monkeys treated with a fusion protein of the HIRMAb and glial derived neurotrophic factor (GDNF).[21] Such small hyperplastic pancreatic lesions are frequently observed at autopsy, and are not pre-malignant.[22] The absence of lesions in the pancreas, or other organs, was also observed following chronic treatment of mice with an IgG-GDNF fusion protein. Mice were treated for 12 weeks with twice-weekly IV injections of a fusion protein of a chimeric monoclonal antibody against the mouse transferrin receptor and GDNF, and no effects on the pathology of pancreas, brain, or other major organs was observed, and no effects of chronic treatment of the IgG-GDNF fusion protein on clinical chemistry was observed.[23] The ADA response against the IgG-GDNF fusion protein was low titer, non-neutralizing, and had no effect on the plasma clearance of the fusion protein following 12 weeks of chronic treatment in mice.[23]

In summary, the present study describes the immune response against an IgG-enzyme fusion protein following 6 months of weekly IV infusions at doses as high as 30 mg/kg. The ADAs do not alter the pharmacokinetics of clearance of the fusion protein from plasma. The absence of an effect on the PK profile over 6 months reduces the likelihood that the ADAs mask any underlying toxicity of the IgG-enzyme fusion protein.

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ABBREVIATIONS

ABST	acetate buffered saline with Tween-80
ADA	anti-drug antibody
AUC	area under the plasma concentration curve
BBB	blood-brain barrier
C_{max}	maximal plasma concentration

C	constant
CHO	Chinese hamster ovary
CL	clearance
DPH	diphenhydramine
ECD	extracellular domain
ERT	enzyme replacement therapy
FcRn	neonatal Fc receptor
GDNF	glial derived neurotrophic factor
GFAP	glial fibrillary acidic protein
GLP	Good Laboratory Practice
HC	heavy chain
HIR	human insulin receptor
HIRMAb	MAb against HIR
HIRMAb-IDUA	fusion protein of HIRMAb and IDUA
ID	injected dose
IDUA	iduronidase
IV	intravenous
LC	light chain
M6PR	mannose 6-phosphate receptor
MAb	monoclonal antibody
MPS	mucopolysaccharidosis
NAb	neutralizing antibody
PK	pharmacokinetics
PTHrP	parathyroid hormone related protein
TCR	tissue cross reactivity
V	variable
$\mathbf{V_{ss}}$	systemic volume of distribution

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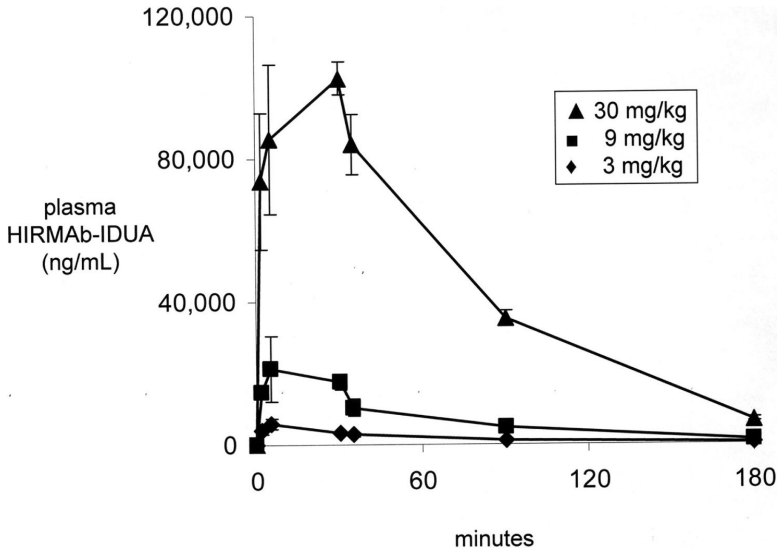

Figure 1.

Plasma immunoreactive HIRMAb-IDUA fusion protein (ng/mL) between 5 and 180 minutes after a 30 minute drug infusion in the low dose (3 mg/kg), mid dose (9 mg/kg) and high dose (30 mg/kg) treatment groups. Data are mean ± SE at each time point. Data are for combined sexes, as there were no significant differences between male and female plasma concentrations.

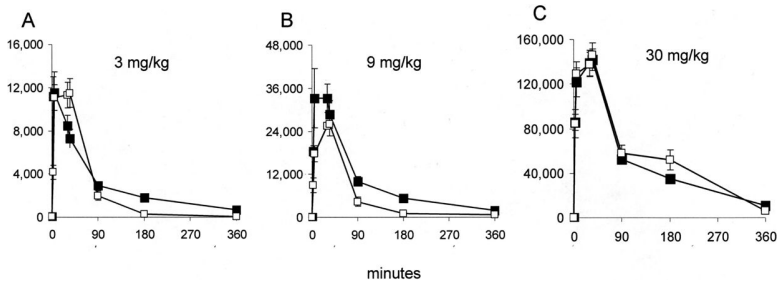

Figure 2.

Plasma IDUA enzyme activity (nmol/hr/mL) between 5 and 360 minutes after a 30 minute infusion of the HIRMAb-IDUA fusion protein in the low dose (3 mg/kg), mid dose (9 mg/kg) and high dose (30 mg/kg) treatment groups. Data are mean ± SE at each time point for combined sexes. There were no significant differences between male and female enzyme activity. Plasma profiles determined at the start of the study (week 1) are shown by solid squares (■), and plasma profiles determined at the end of the study (week 25) are shown by the open squares (□).

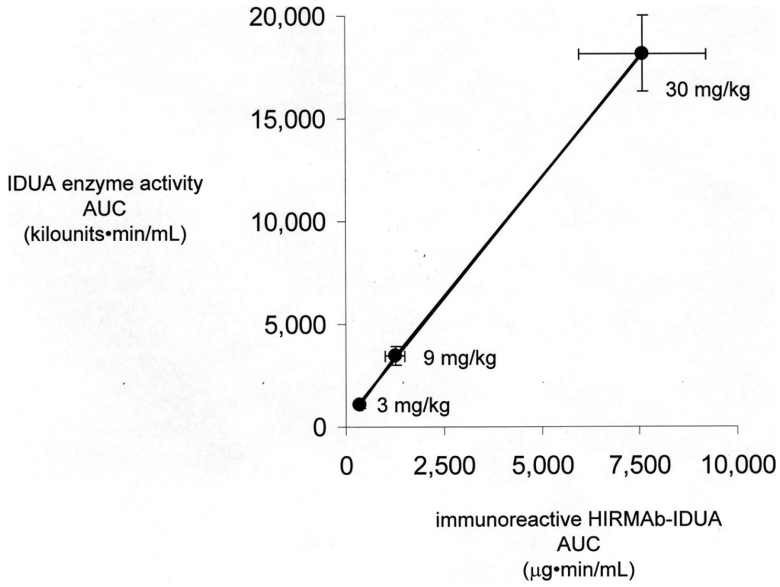

Figure 3.
Comparison of plasma area under the concentration curve (AUC) for the IDUA enzyme activity vs the AUC of immunoreactive HIRMAb-IDUA fusion protein in combined sexes at the start (week 1) of the study.

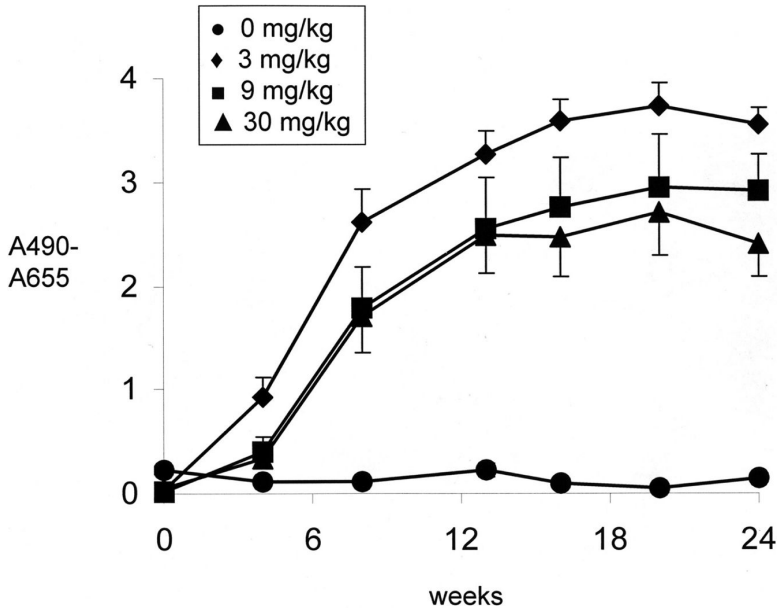

Figure 4.

Time course of ADA formation against the HIRMAb-IDUA fusion protein. Data are mean ± SE (n=8–12 monkeys per time point) for combined sexes. There were no differences between male and female absorbance readings at any time point. A490-A655 is the difference between the absorbance at 490 and 655 nm. The A655, which accounts for light scattering, was <0.05. All plasma samples were diluted 1:50 in PBS.

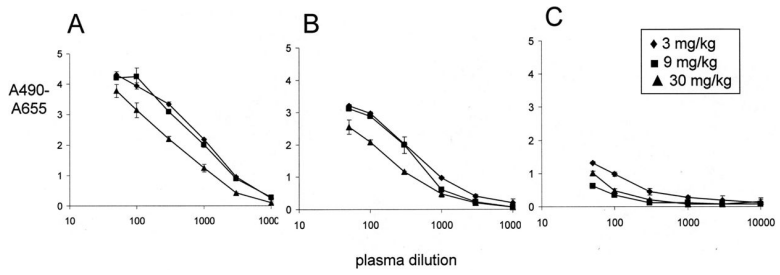

Figure 5.

Absorbance (A490-A655) is plotted against dilution of pools of primate plasma from 1:50 to 1:10,000 in PBS for each of the 3 treatment groups (3 mg/kg, 9 mg/kg, 30 mg/kg) of the HIRMAb-IDUA fusion protein. The pool was produced from the week 20 blood samples. The capture reagent in the sandwich ELISA is the HIRMAb-IDUA fusion protein (panel A), the HIRMAb alone (panel B), or the human IgG1k isotype control (panel C). Data are mean ± SE (n=3 replicates).

Table 1

Pharmacokinetic parameters of plasma clearance of immunoreactive HIRMAb-IDUA fusion protein following a 30 minute IV infusion in male and female Rhesus monkeys at the start (week 1) of the study

Parameter	HIRMAb-IDUA infusion dose		
	3 mg/kg	9 mg/kg	30 mg/kg
C_{max} (μg/mL)	4.56 ± 0.84	16.49 ± 1.51	103.13 ± 5.26
AUC (μg•min/mL)	357 ± 44	1,256 ± 262	7,573 ± 1,628
V_{ss} (mL/kg)	815 ± 153	524 ± 170	291 ± 91
CL (mL/kg/min)	8.42 ± 1.04	7.15 ± 1.52	3.96 ± 0.85
$T_{1/2}$ (min)	67.1 ± 8.4	50.8 ± 10.9	51.1 ± 9.8
Body weight (kg)	3.33 ± 0.39	3.36 ± 0.38	3.31 ± 0.85
Injected dose (mg)	10.0 ± 1.2	30.2 ± 3.4	99.3 ± 10.5

Mean ± SE (n=8–12 monkeys/group). Parameters are for male and female monkeys combined. There were no significant differences between parameters for male and female monkeys at any dose (Student's t-test). Parameters determined from the data in Figure 1.

Table 2

Pharmacokinetic parameters of clearance of IDUA enzyme activity from plasma following a 30 minute IV infusion in combined sexes at start (week 1) and end (week 25) of study

Parameter	Week of study	HIRMAb-IDUA infusion dose		
		3 mg/kg	9 mg/kg	30 mg/kg
C_{max} (kilounits/mL)	1	11.56 ± 1.90	33.19 ± 8.24	141.92 ± 9.39
AUC (kilounits·min/mL)		1,059 ± 145	3,461± 474	18,171 ± 1,896
V_{ss} (mL/kg)		294 ± 62	243 ± 51	182 ± 27
CL (mL/kg/min)		2.25 ± 0.31	2.06 ± 0.28	1.31 ± 0.13
$T_{1/2}$ (min)		89.8 ± 16.0	81.6 ± 13.2	95.9 ± 11.9
Body weight (kg)		3.33 ± 0.39	3.36 ± 0.38	3.31 ± 0.85
Injected dose (kilounits)		7,955 ± 954	24,043 ± 2,706	79,003 ± 8,353
C_{max} (kilounits/mL)	25	11.50 ± 1.32	25.90 ± 3.26	145.89 ± 10.82
AUC (kilounits·min/mL)		862 ± 228	1,624 ± 468	21,412 ± 2,428
V_{ss} (mL/kg)		132 ± 47	257 ± 112	127 ± 19
CL (mL/kg/min)		2.77 ± 0.73	4.41 ± 1.28	1.12 ± 0.13
$T_{1/2}$ (min)		33.1 ± 5.3	40.4 ± 9.4	78.2 ± 7.5
Body weight (kg)		4.12 ± 0.23	3.99 ± 0.13	4.02 ± 0.08
Injected dose (kilounits)		9,845 ± 553	28,584 ± 928	95,974 ± 1,841

Parameters determined from data in Figure 2, where 1 kilounit/mL = 1000 nmol/hr/mL.

Table 3

Immune response titers in Rhesus monkeys treated for 6 months with HIRMAb-IDUA fusion protein

Parameter	Capture agent		
	HIRMAb-IDUA	HIRMAb	hIgG1k
Titer at 3 mg/kg	10.1 ± 0.2	6.1 ± 0.2	1.4 ± 0.3
Titer at 9 mg/kg	9.3 ± 0.1	6.0 ± 0.8	0.33 ± 0.10
Titer at 30 mg/kg	6.6 ± 0.3	3.5 ± 0.1	0.61 ± 0.08
Average titer, all doses	8.7 ± 1.8	5.2 ± 1.5	0.78 ± 0.55
Titer relative to HIRMAb-IDUA titer	100 %	60 %	9 %

Date are mean ± SD. Immune response titers determined from data in Figure 5.