Glycemic Control and Chronic Dosing of Rhesus Monkeys with a Fusion Protein of Iduronidase and a Monoclonal Antibody Against the Human Insulin Receptor

Ruben J. Boado, Eric Ka-Wai Hui, Jeff Zhiqiang Lu, and William M. Pardridge

Department of Medicine, University of California Los Angeles, Los Angeles, California (R.J.B., W.M.P.); and, ArmaGen Technologies, Inc., Santa Monica, California (R.J.B., E.K.-W.H., J.Z.L.)

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ABSTRACT:

Hurler's syndrome, or mucopolysaccharidosis type I, is a lysosomal storage disorder caused by mutations in the gene encoding the lysosomal enzyme iduronidase (IDUA). The disease affects both peripheral tissues and the central nervous system (CNS). Recombinant IDUA treatment does not affect the CNS, because IDUA does not cross the blood-brain barrier (BBB). To enable BBB penetration, human IDUA was re-engineered as an IgG-IDUA fusion protein, where the IgG domain is a genetically engineered monoclonal antibody (MAb) against the human insulin receptor (HIR). The HIRMAb penetrates the brain from the blood via transport on the endogenous BBB insulin receptor and acts as a molecular Trojan horse to deliver the fused IDUA to the brain. Before human testing, the HIRMAb-IDUA fusion protein was evaluated in

a 6-month weekly dosing toxicology study at doses of 0, 3, 9, and 30 mg/kg/week of the fusion protein administered to 40 rhesus monkeys. The focus of the present study is the effect of chronic high dose administration of this fusion protein on plasma glucose and long-term glycemic control. The results show that the HIRMAb has weak insulin agonist activity and causes hypoglycemia at the high dose, 30 mg/kg, after intravenous infusion in normal saline. When dextrose is added to the saline infusion solution, no hypoglycemia is observed at any dose. An intravenous glucose tolerance test performed at the end of the 6 months of chronic treatment showed no change in glucose tolerance at any dose of the HIRMAb-IDUA fusion protein.

Introduction

Mucopolysaccharidosis Type I, also called MPSI, is a lysosomal storage disorder caused by mutations in the gene encoding the lysosomal enzyme α -L-iduronidase (IDUA). MPSI patients are treated with enzyme replacement therapy (Brady and Schiffmann, 2004) and weekly intravenous infusions of the recombinant IDUA enzyme. However, many patients with MPSI have Hurler's syndrome, where the disorder also affects the central nervous system (CNS). Enzyme replacement therapy is not effective for the CNS (Wraith, 2001), because IDUA, a large molecule drug, does not cross the blood-brain barrier (BBB) and does not penetrate the brain from the blood (Miebach, 2005).

Recombinant enzymes such as IDUA can be re-engineered as BBB-penetrating pharmaceuticals by the fusion of IDUA to a genetically engineered monoclonal antibody (MAb) against the human insulin receptor (HIR) (Boado et al., 2008). The insulin receptor is expressed at the human BBB (Pardridge et al., 1985). The HIRMAb binds the BBB insulin receptor and acts as a molecular Trojan horse to ferry the fused IDUA enzyme across the BBB. The insulin receptor

also is expressed on brain cells, and the HIRMAb also mediates endocytosis into target cells, where the fusion protein then is triaged to the lysosomal compartment of the cell (Boado et al., 2008).

Treatment of Hurler's syndrome with HIRMAb-IDUA fusion proteins requires chronic dosing with weekly intravenous infusions of the HIRMAb-IDUA fusion protein. Under these conditions, it is possible that the HIRMAb domain of the fusion protein could have either agonist or antagonist properties at the HIR, causing either hypoglycemia or hyperglycemia, respectively. The HIRMAb cross-reacts with the insulin receptor in Old World primates such as the rhesus monkey but does not cross-react with the insulin receptor of lower animals or even New World primates (Pardridge et al., 1995). Therefore, chronic dosing studies must be performed in the rhesus monkey. In a prior study, rhesus monkeys were treated twice weekly for 4 weeks with 0.2, 2, and 20 mg/kg doses of the HIRMAb-IDUA fusion protein (Boado et al., 2009). No evidence of hypo- or hyperglycemia was observed at these doses of the HIRMAb-IDUA fusion protein (Boado et al., 2009). In the present investigation, the dose of the HIRMAb-IDUA fusion protein is increased to 3, 9, and 30 mg/kg/dose, and the duration of the weekly dosing is increased to 6 months. Glucose concentrations in plasma and cerebrospinal fluid (CSF) are measured at each dose of the fusion protein. Glycemic control at the end of the study is evaluated with intravenous glucose tolerance tests.

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ABBREVIATIONS: BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; HIR, human insulin receptor; HIRMAb, MAb against HIR; IDUA, iduronidase; HIRMAb-IDUA, fusion protein of HIRMAb and IDUA; MAb, monoclonal antibody.

Materials and Methods

Production of the HIRMAb-IDUA Fusion Protein. The HIRMAb-IDUA fusion protein, also called AGT-181, is a heterotetrameric protein comprised of two heavy chains and two light chains; an IDUA protein is fused to the C terminus of each heavy chain (Boado et al., 2008). The HIRMAb-IDUA fusion protein was produced in Chinese hamster ovary cells in serum-free medium in a 50-L perfusion mode Wave bioreactor (GE Healthcare Life Sciences, Pittsburgh, PA) as described previously (Boado et al., 2009). The conditioned medium was clarified by depth filtration, and the fusion protein was purified with three columns: a 1.8-L protein A column (MAB Select; GE Healthcare Life Sciences), a 1.8-L cation exchange column (SP Sepharose; GE Healthcare Life Sciences), and a 1.2-L anion exchange column (Q Sepharose; GE Healthcare Life Sciences) followed by nanofiltration and diafiltration in the final diluent, which is 10 mM sodium acetate/140 mM NaCl (pH 5.5)/0.001% Tween 80. The purity, identity, and potency of the fusion protein were verified by SDS-polyacrylamide gel electrophoresis, size exclusion chromatography, human IgG and IDUA Western blot analysis, HIR receptor binding assay, IDUA fluorometric enzymatic assay, host cell protein impurity, protein A impurity, host cell DNA impurity, endotoxin, and subvisible particles. The identity of the protein was confirmed by peptide mapping with liquid chromatography-tandem mass spectrometry at The Scripps Research Institute (La Jolla, CA). The final formulation contained 5 mg/ml fusion protein, which was stored upright as a sterile liquid at 4°C. Stability studies, based on eight analytical methods on purity, identity, potency, and sterility, showed that the protein was stable at this formulation for 2 years.

Chronic Dosing of Rhesus Monkeys. Juvenile rhesus monkeys (*Macaca mulatta*) of mixed sex (20 males, 20 females) were used for all of the studies and were housed at MPI Research, Inc. (Mattawan, MI) in stainless steel cages in a controlled environment (18–28°C and 30–70% relative humidity) on a 12-h light/dark cycle. LabDiet Certified Primate Diet (PMI Nutrition International, Crystal, MN) was provided twice daily. Animals were fasting before all of the study drug infusions, as food was withheld the morning before drug infusion. Tap water was provided *ad libitum*. All of the aspects of the primate study performed at MPI Research, Inc. were conducted in strict compliance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58. All of the procedures were in compliance with the Animal Welfare Act Regulations and were approved by the Institutional Animal Care and Use Committee.

The primates were treated with 0 mg/kg (6 males, 6 females), 3 mg/kg (4 males, 4 females), 9 mg/kg (4 males, 4 females), or 30 mg/kg (6 males, 6 females) of the HIRMAb-IDUA fusion protein administered as an intravenous infusion over a 30-min period in 50 ml of either normal saline or 10% dextrose in normal saline. In the 26-week toxicity study, the doses were administered every 7 days for 26 consecutive weeks. The HIRMAb-IDUA fusion protein was administered on a weekly basis, because this parallels the current standard practice in the treatment of Hurler's syndrome, which uses weekly intravenous infusions of the recombinant IDUA (Wraith, 2001; Miebach, 2005). For study drug infusion at week 1 and week 25, blood was removed from the femoral vein and collected in tubes with K₂-EDTA at 0, 2, 5, 30, 35, 90, min and 3, 6, and 23 h after the start of the 30-min intravenous infusion of the HIRMAb-IDUA fusion protein. The blood was separated into plasma, which then was stored at –70°C until analysis. Fasting plasma glucose was measured monthly during the study. During the first week, CSF was removed via the cisterna magna at 0, 3, and 23 h after the intravenous infusion of the HIRMAb-IDUA fusion protein. Fasting plasma glucose was measured in blood removed at weeks 0, 4, 8, 13, 16, 20, and 24.

Glucose Assays. Plasma glucose was determined at MPI Research, Inc. with an Olympus AU2700 chemistry analyzer (Olympus America, Inc., Center Valley, PA). CSF glucose was determined with a glucose assay kit from BioVision (Mountain View, CA) using a spectrophotometric method and absorbance at 570 nm. The assay is run in 96-well plates with a standard curve of D-glucose of 0 to 10 nmol/well and 50-μl samples per well of a 1:50 dilution of primate CSF.

Intravenous Glucose Tolerance Test. The HIRMAb-IDUA fusion protein was infused in 50 ml of 10% dextrose/normal saline over 30 min, and plasma glucose was measured at 0, 2, 5, 30, 35, 60, 90, 180, and 360 min after the start of the infusion. The plasma glucose, $A(t)$, was plotted versus

the time (t) of infusion between 30 and 90 min and fit to the equation $\ln[A(t)] = \ln(A_{\max}) - kt$, where $A_{\max}$ is the maximal plasma glucose at zero time and k is the rate constant of glucose clearance from the plasma. The half-time of plasma glucose clearance, $T_{1/2}$, was determined from $\ln(2)/k$. The intravenous glucose tolerance test was performed on fasting monkeys with the end of study (week 25) drug infusion.

Statistical Analysis. Data are reported as mean $\pm$ S.E., and statistically significant differences were assessed at the $P < 0.05$ level using analysis of variance with Bonferroni correction.

Results

The HIRMAb-IDUA fusion protein was infused intravenously over 30 min in 50 ml of normal saline in rhesus monkeys at four doses (0, 3, 9, and 30 mg/kg). Plasma glucose was measured at 0, 2, 5, 30, 35, 90, 180, 360, and 1380 min after the start of the 30-min infusion, and the values are reported in Table 1 by either sex or combined sexes. There are no significant differences between sexes at any time point (Table 1). The glucose in the fusion protein-treated animals (combined sexes) was 20 to 29% lower than that of the saline controls at 30 and 35 min after start of the 30-min drug infusion at all three doses of the fusion protein, with no dose relationships (Table 1). The plasma glucose is decreased 31 and 47% at 90 and 180 min only in the high dose group, 30 mg/kg (Table 1). Figure 1 shows the plasma glucose for individual monkeys in the saline group and in the high dose group (30 mg/kg). One monkey in the saline group and four monkeys in the 30 mg/kg group have plasma glucose values of <40 mg%, and the nadir is at 180 min after the start of the 30-min infusion (Fig. 1). The level of hypoglycemia in two monkeys at 180 min was severe, with plasma glucose values of 11 mg% after the intravenous infusion of the HIRMAb-IDUA fusion protein in normal saline at a dose of 30 mg/kg (Fig. 1).

During the last week of the 26-week treatment study, the HIRMAb-IDUA fusion protein was formulated in 50 ml of 10% dextrose/normal

TABLE 1
Plasma glucose at start of study

Data are in mg/dl. Mean $\pm$ S.E. ($n = 8$ –12 per group in combined sexes and 4–6 per group in male or female groups).

Sex	Minutes	HIRMAb-IDUA Injection Dose			
		0 mg/kg	3 mg/kg	9 mg/kg	30 mg/kg
Combined	0	83 $\pm$ 7	74 $\pm$ 4	70 $\pm$ 5	78 $\pm$ 4
	2	73 $\pm$ 3	67 $\pm$ 7	67 $\pm$ 3	75 $\pm$ 4
	5	74 $\pm$ 4	64 $\pm$ 7	64 $\pm$ 3	74 $\pm$ 4
	30	75 $\pm$ 3	55 $\pm$ 6 [†]	57 $\pm$ 4*	60 $\pm$ 3*
	35	79 $\pm$ 5	56 $\pm$ 5 [†]	59 $\pm$ 4*	59 $\pm$ 3 [†]
	90	91 $\pm$ 5	75 $\pm$ 5	72 $\pm$ 4	63 $\pm$ 6 [†]
	180	74 $\pm$ 8	58 $\pm$ 5	61 $\pm$ 3	39 $\pm$ 5 [†]
	360	74 $\pm$ 5	72 $\pm$ 12	79 $\pm$ 11	65 $\pm$ 8
	1380	76 $\pm$ 5	79 $\pm$ 8	69 $\pm$ 5	70 $\pm$ 8
	0	75 $\pm$ 7	81 $\pm$ 6	72 $\pm$ 6	79 $\pm$ 7
Male	2	75 $\pm$ 4	78 $\pm$ 5	65 $\pm$ 7	70 $\pm$ 4
	5	76 $\pm$ 4	74 $\pm$ 6	62 $\pm$ 5	69 $\pm$ 4
	30	74 $\pm$ 3	58 $\pm$ 2	56 $\pm$ 6*	57 $\pm$ 3*
	35	75 $\pm$ 4	60 $\pm$ 3	59 $\pm$ 7	58 $\pm$ 3*
	90	86 $\pm$ 5	81 $\pm$ 6	75 $\pm$ 4	62 $\pm$ 4 [†]
	180	65 $\pm$ 9	69 $\pm$ 4	59 $\pm$ 6	37 $\pm$ 6 [†]
	360	66 $\pm$ 4	58 $\pm$ 5	89 $\pm$ 10	70 $\pm$ 14
	1380	75 $\pm$ 10	89 $\pm$ 8	64 $\pm$ 5	70 $\pm$ 12
	0	90 $\pm$ 13	68 $\pm$ 5	69 $\pm$ 9	77 $\pm$ 6
	2	70 $\pm$ 5	55 $\pm$ 9	69 $\pm$ 3	80 $\pm$ 7
Female	5	72 $\pm$ 7	54 $\pm$ 12	67 $\pm$ 3	79 $\pm$ 7
	30	77 $\pm$ 6	52 $\pm$ 12	59 $\pm$ 4	63 $\pm$ 6
	35	82 $\pm$ 9	52 $\pm$ 11	60 $\pm$ 5	61 $\pm$ 6
	90	96 $\pm$ 8	69 $\pm$ 9	70 $\pm$ 7	63 $\pm$ 11
	180	84 $\pm$ 12	47 $\pm$ 7	64 $\pm$ 2	41 $\pm$ 9*
	360	82 $\pm$ 9	87 $\pm$ 22	68 $\pm$ 20	61 $\pm$ 9
	1380	76 $\pm$ 5	69 $\pm$ 12	75 $\pm$ 8	69 $\pm$ 13

* $P < 0.05$ difference from 0 mg/kg by analysis of variance.

[†] $P < 0.01$ difference from 0 mg/kg by analysis of variance.

FIG. 1. Plasma glucose is plotted versus time after the start of a 30 min infusion of the HIRMAb-IDUA fusion protein at a dose of either 0 mg/kg (left) or 30 mg/kg (right). Data are shown for individual monkeys (6 males and 6 females in each treatment group). The HIRMAb-IDUA fusion protein is infused in 50 ml of normal saline with no glucose supplement at the start (week 1) of the study. The horizontal bar defines a plasma glucose of 40 mg%, which is a minimum value for all but one of the saline-infused monkeys.

saline and infused over 30 min in the same group of rhesus monkeys at doses of 0, 3, 9, and 30 mg/kg. No hypoglycemia was observed in any animal, and the plasma glucose values are shown in Fig. 2. The plasma glucose peaked at the end of the 30 min of fusion protein infusion (Fig. 2). The rate of decline in the plasma glucose was evaluated by linear regression analysis to produce the half-time ($T_{1/2}$) of glucose clearance from plasma for each of the four treatment doses of the HIRMAb-IDUA fusion protein. There were no differences in the $T_{1/2}$ among all of the treatment groups, and glucose was cleared from plasma with a $T_{1/2}$ of 32 to 35 min (Table 2).

Plasma glucose was measured monthly in all of the monkeys before the intravenous infusion of the study drug, and the plasma glucose values are shown in Table 3 by sex and for combined groups. There are no sex differences and no upward or downward trends in plasma glucose over the course of 24 weeks.

Glucose was measured in CSF at 0, 3, and 23 h after the 30-min infusion of HIRMAb-IDUA fusion protein at each of the four doses (0, 3, 9, and 30 mg/kg) in normal saline. The only significant difference in CSF glucose was a 48% decrease at 3 h in the 30 mg/kg treatment group (Fig. 3). The CSF glucose, at 3 h after drug infusion, in all 40 monkeys in the study correlated with the plasma glucose, at 3 h after drug infusion, and the average CSF/plasma glucose ratio was 54% for all of the monkeys (Fig. 4). There were no significant differences in the glucose concentrations in CSF at 0 and 23 h in any of the four treatment groups. The CSF glucose at 0 h was 63 ± 16 , 69 ± 20 , 58 ± 6 , and 62 ± 12 mg% and at 23 h was 54 ± 9 , 67 ± 18 , 52 ± 8 , and 63 ± 16 mg%, respectively, after the administration of 0, 3, 9, and 23 mg/kg HIRMAb-IDUA fusion protein (mean $\pm$ S.D.).

Discussion

The results of the studies are consistent with the following conclusions. First, high doses, 30 mg/kg, of the HIRMAb-IDUA fusion protein in fasting rhesus monkeys cause hypoglycemia with a nadir of 39 ± 5 mg% at 180 min after a 30-min infusion of the fusion protein in 50 ml of normal saline (Table 1). Second, the hypoglycemia can be severe in some monkeys, because the nadir was as low as 11 mg% in two monkeys at the 30 mg/kg dose of the fusion protein (Fig. 1). Third, the hypoglycemia is eliminated by the inclusion of glucose in the infusion solution (Fig. 2). Fourth, the rate of clearance of glucose from plasma, which is a measure of glucose tolerance, is unchanged in all of the treatment groups at the end of the 26 weeks of fusion protein dosing (Fig. 2), and the half-time of glucose clearance at all of the doses is the same, 32 to 35 min (Table 2). Fifth, there is no evidence of impaired glucose tolerance with chronic fusion protein treatment, because the monthly fasting plasma glucose is unchanged in all of the treatment groups over the course of the 6 months of treatment (Table 3). Sixth, the CSF glucose is decreased at 3 h after

intravenous infusion of 30 mg/kg of the HIRMAb-IDUA fusion protein in normal saline, and the CSF glucose parallels the corresponding plasma glucose in each monkey (Fig. 4).

A monoclonal antibody against the α subunit of the human insulin receptor may have either agonist or antagonist properties. Antibodies against the insulin receptor that demonstrate agonist properties cause an increase in glucose uptake by cells (Brunetti et al., 1989), which is associated with an increase in glucose clearance from plasma (Bhaskar et al., 2012). The HIRMAb domain of the HIRMAb-IDUA fusion protein shows agonist properties, albeit only at the highest treatment dose of 30 mg/kg. At this dose, hypoglycemia is induced, which peaks at 3 h after a 30-min infusion of the HIRMAb-IDUA fusion protein in normal saline (Fig. 1; Table 1). The hypoglycemia is eliminated when glucose is added to the fusion protein infusion solution (Fig. 2). An insulin receptor antibody with antagonist action can cause hyperglycemia and impaired glucose tolerance (Malek et al., 2010). The HIRMAb domain of the HIRMAb-IDUA fusion protein exhibits no antagonist properties, because fasting hyperglycemia is not induced (Table 3) and the rate constant of glucose clearance from plasma is unchanged (Table 2) after 6 months of weekly dosing of the fusion protein at doses of 3, 9, or 30 mg/kg. The lack of an effect of chronic treatment with the HIRMAb fusion protein on glycemic control is not due to a change in the exposure over the course of 6 months of treatment. A pharmacokinetics analysis shows that there is no change in the rate of clearance of the HIRMAb-IDUA fusion protein from the blood at the start (week 1) and end (week 25)

FIG. 2. Plasma glucose is plotted versus time after the start of a 30-min infusion of the HIRMAb-IDUA fusion protein at a dose of 0, 3, 9, or 30 mg/kg. The HIRMAb-IDUA fusion protein is infused in 50 ml of 10% dextrose/normal saline at the end (week 25) of the study. The 23-h plasma glucose was 80 ± 4 , 80 ± 5 , 78 ± 7 , and 80 ± 4 mg% for the 0, 3, 9, and 30 mg/kg treatment groups, respectively. Data are mean $\pm$ S.E. ($n = 6$ –12 combined sexes in each group).

TABLE 2

Intravenous glucose tolerance test at end of 26-week dosing

Parameters were determined by nonlinear regression analysis of the plasma glucose between 30 and 90 min after a 30-min infusion of 10% glucose. Data are means $\pm$ S.E. for combined sexes. $T_{1/2}$ = half-time of glucose clearance from blood after termination of the glucose infusion.

Parameter	Units	HIRMAb-IDUA Dose			
		0 mg/kg	3 mg/kg	9 mg/kg	30 mg/kg
k	min^{-1}	0.020 ± 0.001	0.022 ± 0.003	0.022 ± 0.002	0.021 ± 0.002
$T_{1/2}$	min	35 ± 2	32 ± 4	32 ± 3	33 ± 2

of the 6 months of treatment (R. J. Boado, E. K.-W. Hui, J. Z. Lu, and W. M. Pardridge, manuscript in preparation).

The concentration of glucose in CSF also is reduced at 3 h after intravenous infusion of the HIRMAb-IDUA fusion protein at a dose of 30 mg/kg in normal saline (Fig. 3). Insulin does not affect glucose uptake by the brain (Hasselbalch et al., 1999), and the CSF glucose is regulated by the plasma glucose concentration. The direct relationship between CSF and plasma glucose is demonstrated in this study (Fig. 4), and the mean CSF/plasma glucose ratio is 0.54 (Fig. 4). This value is in agreement with the CSF/plasma ratio reported in either humans or rhesus monkeys, which is 0.5 to 0.6 (Powers, 1981; Davis et al., 1993). Therefore, high doses of the HIRMAb-IDUA fusion protein have no direct effect on glucose distribution in CSF, and CSF glucose concentrations parallel the corresponding concentrations of glucose in plasma. Reductions in plasma and CSF glucose are observed at 3 h after HIRMAb-IDUA infusion only at the high dose of 30 mg/kg. Reductions in glucose in either plasma or CSF are not observed after 3 or 9 mg/kg doses of the fusion protein. Because the projected therapeutic dose of the HIRMAb-IDUA fusion protein is 0.6 to 1 mg/kg (Boado et al., 2008, 2011), no alterations in plasma or CSF glucose are expected in humans.

The effect of high doses of the HIRMAb-IDUA fusion protein on plasma glucose is transient, because the plasma glucose increases between 180 and 360 min after the infusion (Fig. 1). The rapid reversal of the plasma glucose is consistent with the rapid removal of the HIRMAb-IDUA fusion protein from plasma, owing to uptake by peripheral tissues. At a dose of 20 mg/kg in rhesus monkeys, the HIRMAb-IDUA fusion

protein is cleared rapidly from plasma with a systemic clearance of 3.3 ± 0.4 ml/min/kg and a high systemic volume of distribution of 393 ± 75 ml/kg (Boado et al., 2009). The mean residence time of the fusion protein in the circulation in rhesus monkeys is 121 ± 24 min (Boado et al., 2009). Therefore, the return to normoglycemia by 6 h after drug infusion (Table 1) is consistent with nearly complete removal of the fusion protein from the circulation.

Lower doses of the HIRMAb-IDUA fusion protein do not cause hypoglycemia. In a previous study with a 20 mg/kg dose of the HIRMAb-IDUA fusion protein in rhesus monkeys, no reduction in plasma or CSF glucose was observed (Boado et al., 2009). In another study, a HIRMAb fusion protein was administered to rhesus monkeys by bolus intravenous injection at 10 mg/kg every 12 h for five consecutive doses, and no hypoglycemia was observed (Pardridge and Boado, 2009). In the present study, there are only modest reductions in glucose at 0 to 5 min after the termination of the 30-min infusion and no significant reductions in plasma glucose at 90 to 1380 min after the start of the 30-min intravenous infusion of 3 or 9 mg/kg doses of the HIRMAb-IDUA fusion protein in normal saline (Table 1). The 3 or 9 mg/kg doses are higher than the expected therapeutic dose of the HIRMAb-IDUA fusion protein, which may be on the order of 1 mg/kg. The CNS lysosomal inclusion bodies are reduced in Hurler mice after chronic treatment with the mouse homolog of the HIRMAb-IDUA fusion protein, and the treatment dose is 1 mg/kg of fusion protein intravenously (Boado et al., 2011). The level of IgG-enzyme penetration of the brain after intravenous administration is comparable in mice and rhesus monkeys, where the brain uptake in either species is approximately 1% of the injected dose per brain (Boado et al., 2008; Zhou et al., 2012). On the basis of the brain uptake in the primate of 1% of the injected dose per brain and the endogenous IDUA enzyme activity in the human brain, a treatment dose of 0.6 mg/kg is predicted to replace >50% of the IDUA enzyme activity in the brain (Boado et al., 2008).

In conclusion, these studies show that the HIRMAb domain of the HIRMAb-IDUA fusion protein has weak insulin agonist properties

TABLE 3

Plasma glucose by week of study

Data are in mg/dl. Mean $\pm$ S.E. ($n = 8$ –12 per group in combined sexes and 4–6 per group in male or female groups). Blood was removed for plasma glucose 1 week after the previous dosing of the study drug.

Sex	Weeks	HIRMAb-IDUA Injection Dose			
		0 mg/kg	3 mg/kg	9 mg/kg	30 mg/kg
Combined	0	83 ± 7	74 ± 4	70 ± 5	78 ± 5
	4	77 ± 2	77 ± 5	79 ± 7	72 ± 3
	8	86 ± 4	79 ± 4	78 ± 7	79 ± 3
	13	77 ± 3	76 ± 4	77 ± 4	78 ± 6
	16	76 ± 3	70 ± 5	70 ± 6	79 ± 6
	20	72 ± 5	68 ± 5	71 ± 5	78 ± 5
Male	24	73 ± 2	72 ± 5	81 ± 7	80 ± 4
	0	75 ± 7	81 ± 6	72 ± 6	79 ± 7
	4	79 ± 3	81 ± 4	84 ± 12	67 ± 5
	8	88 ± 6	81 ± 2	79 ± 8	83 ± 7
	13	76 ± 2	79 ± 3	76 ± 6	76 ± 6
	16	80 ± 6	73 ± 3	72 ± 9	74 ± 6
Female	20	77 ± 7	65 ± 4	71 ± 7	75 ± 4
	24	72 ± 1	67 ± 4	87 ± 15	76 ± 4
	0	90 ± 13	68 ± 5	69 ± 9	77 ± 6
	4	76 ± 2	73 ± 10	74 ± 7	77 ± 5
	8	84 ± 6	77 ± 9	78 ± 12	76 ± 3
	13	78 ± 6	73 ± 8	77 ± 7	81 ± 11
	16	73 ± 2	69 ± 9	68 ± 8	84 ± 10
	20	67 ± 8	71 ± 9	70 ± 6	81 ± 11
	24	74 ± 4	76 ± 9	76 ± 6	84 ± 7

FIG. 3. CSF glucose at 0, 3, and 23 h after HIRMAb-IDUA fusion protein infusion is shown for 0, 3, 9, and 30 mg/kg doses of the HIRMAb-IDUA fusion protein. Data are mean $\pm$ S.E. ($n = 8$ –12 combined sexes in each group). The HIRMAb-IDUA fusion protein is infused in 50 ml of normal saline at the start (week 1) of the study. *, $P < 0.05$ difference from control (0 mg/kg) as determined by analysis of variance.

FIG. 4. CSF glucose is plotted versus the corresponding plasma glucose at 3 h after the intravenous infusion of the HIRMAb-IDUA fusion protein for all four treatment groups (0, 3, 9, and 30 mg/kg). Data for individual monkeys is shown. The slope was determined by linear regression analysis. CSF and plasma glucose were determined after HIRMAb-IDUA infusion during the first week of the study.

that are observed only at the highest dose of 30 mg/kg, which may be as much as 30-fold greater than planned therapeutic doses of the fusion protein. Hypoglycemia is not observed after the administration of doses lower than 30 mg/kg. These observations are corroborated by a prior study, which showed no hypoglycemia after intravenous infusion of the HIRMAb-IDUA fusion protein in saline at doses up to 20 mg/kg in rhesus monkeys (Boado et al., 2009). Any concern about hypoglycemia is mitigated by simply adding dextrose to the saline infusion of drug. The present study shows that a 10% dextrose additive is not necessary, because this dose of glucose causes transient hyperglycemia (Fig. 2). A preferred formulation is normal saline with 5% dextrose for routine administration of HIRMAb-derived fusion proteins.

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Authorship Contributions

Participated in research design: Boado and Pardridge.
Conducted experiments: Boado, Hui, and Lu.

Contributed new reagents or analytic tools: Boado.

Performed data analysis: Boado, Hui, Lu, and Pardridge.

Wrote or contributed to the writing of the manuscript: Boado, Hui, Lu, and Pardridge.

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Address correspondence to: Dr. William M. Pardridge, Department of Medicine, University of California Los Angeles, Warren Hall 13-164, 900 Veteran Ave., Los Angeles, CA 90024. E-mail: wpardridge@mednet.ucla.edu