A GH receptor antisense oligonucleotide inhibits hepatic GH receptor expression, IGF-I production and body weight gain in normal mice

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Abstract

Diabetic retinopathy and acromegaly are diseases associated with excess action of GH and its effector IGF-I, and there is a need for improved therapies. We have designed an optimised 2'-O-(2-methoxyethyl)-modified phosphorothioate oligodeoxynucleotide, ATL 227446, and demonstrated its ability to suppress GH receptor mRNA in vitro. Subcutaneous injections of ATL 227446 reduced GH receptor mRNA levels, GH binding activity and serum IGF-I levels in mice after seven days of dosing. The reduction in serum IGF-I could be sustained for over ten weeks of dosing at therapeutically relevant levels, during which there was also a significant decrease in body weight gain in antisense-treated mice relative to saline and mismatch control-treated mice. The findings indicate that administration of an antisense oligonucleotide to the GH receptor may be applicable to human diseases in which suppression of GH action provides therapeutic benefit.

Introduction

Growth hormone (GH) expression or signalling has been indicated in the pathogenesis of several diseases including acromegaly and diabetic retinopathy, where GH manipulation with drug treatment is an accepted mode of therapy. Varying approaches include blocking responsiveness to GH through receptor antagonism (pegvisomant), or blocking secretion of GH from the pituitary using somatostatin or its analogues (octreotide). This report describes a novel strategy for the manipulation of GH signalling and subsequent action, using an antisense oligonucleotide (ASO) that specifically reduces expression of the growth hormone receptor (GHR).

Since the major origin of circulating insulin-like growth factor-I (IGF-I) is the GH-stimulated hepatocytes and as the liver is also a major site of ASO accumulation following systemic delivery (Crooke 2004), targeting the growth hormone receptor with ASO represents an attractive therapeutic prospect. While initial approaches to antisense therapy were problematic, recent advances in oligonucleotide chemistry have resulted in the production of second and third generation molecules with enhanced safety, efficacy and stability in vivo. There are currently some twenty antisense drugs in clinical development for a range of indications (reviewed in Crooke 2004) that have activity in vivo following administration in the absence of lipophilic agents (Zhang et al. 2000, Crooke et al. 2005).

Inhibition of GH signalling results in suppression of IGF-I secretion from liver tissues (Rowland et al. 2005). Serum IGF-I is required for mediation of many of the indirect effects of growth hormone action and thus acts as a surrogate measure of bioactive GH (Cummings & Merriam 2003). Direct measurement of GH in humans is problematic due to the pulsatile nature of GH release. Consequently, the level of serum IGF-I, which is more stable throughout the day, is the most widely used parameter for measurement of the bioactivity of GH in humans (Monson 2001). In acromegaly, normalisation of serum IGF-I levels is the currently accepted definition of cure (Melmed et al. 2002). Similarly, reduction in serum IGF-I induced by administration of the somatostatin analogue, octreotide, significantly reduced the need for photocoagulation therapy in diabetic retinopathy patients (Grant et al. 2000).

This report describes the selection of ATL 227446, a 5’–10–5’ 2’-O-(2-methoxyethyl) (2’-MOE) modified ASO directed to the mouse GHR, through the use of...
mRNA knockdown assay in vitro, and the subsequent demonstration of in vivo knockdown of GHR mRNA in normal mice. Reduced binding of growth hormone to the liver cells of mice treated with this compound is shown and the effect of the ASO on serum IGF-I levels after either one or ten weeks of treatment is demonstrated. Reduced body weight gain in ASO-treated mice is also demonstrated in the ten-week study.

Materials and Methods

Antisense oligonucleotides

A panel of ASOs to mouse GHR and control oligonucleotides were prepared by Isis Pharmaceuticals (Carlsbad, CA, USA). ASOs were phosphorothioate throughout with 2′-O-(2-methoxyethyl) (2′-MOE) modifications in the five outer 5′ and 3′ positions. Control oligonucleotides CoA (TTCTACTCGCAGGATTTAC), CoB (GAGGTCTCGACTTACCCCGT), CoC (AGAGGC TACCTAATACAA) and CoD (TTACCGTATGGTT CCTCAC), with sequences non-specific to GHR, were prepared. These controls were also phosphorothioate throughout with 2′-MOE modifications (underlined) in the five outer 5′ and 3′ positions. The 2′-MOE modification imparts improved safety, efficacy and in vivo stability, whilst the unmodified portion of the sequence is maintained to allow RNase H activity. Controls CoA and CoB are standard control oligonucleotides used in in vitro oligonucleotide selection, while controls CoC and CoD were developed following selection of the optimised sequence. They represent a scrambled version of ATL 227446 and a completely random oligonucleotide sequence respectively. These two latter controls were used in in vitro studies.

Oligonucleotide treatment in cell culture

Mouse brain endothelial (b.END-3) cells were seeded in a 96-well plate format at a density of 5000 cells per well, and allowed to adhere overnight for a confluence of 75% at the time of transfection. Lipofectin (Invitrogen Corp) was diluted in Opti-MEM (Invitrogen Corp) and incubated at room temperature for 30 min, as per the manufacturer’s instructions. Oligonucleotides were diluted in the Opti-MEM/lipofectin mix at a final ratio of 2·5 µl lipofectin/100 nM oligo/1 ml Opti-MEM. Growth medium was removed and the formulated oligonucleotides were added directly to the cells. Four hours later the cells were re-fed with complete growth medium. Cells were harvested for total RNA isolation 16 h post-transfection with a commercial lysis buffer, RLT (Qiagen).

Animals

All animal studies were conducted in accordance with the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes, and were approved by the University of Queensland Animal Bioethics Committee. Weanling BALB/c male mice (approximately 3 weeks of age) used for all studies were purchased from the Animal Resources Centre, Perth, WA, Australia, and housed at The University of Queensland for the duration of the studies. Temperature was maintained at 22–24 °C on a 12-h light cycle and food pellets and water were available ad libitum.

For all in vivo studies, mice were allowed to adjust to their environment for one week prior to commencing the study. Groups of mice were selected to begin the trial at the same weight (11 ± 0·3 g).

Experiment 1 To determine the in vivo effects of ATL 227446 on GHR mRNA expression levels, groups of ten mice were injected every second day over seven days (for a total of three doses) with ATL 227446 at 30 mg/kg or 50 mg/kg, or with the control oligonucleotide CoC at 50 mg/kg, or with saline (vehicle control). Drugs were administered s.c. to the nape of the neck in a volume of 200 µl. At day seven, mice were killed and liver tissue was collected for GHR mRNA analysis.

Experiment 2 To assess the effect of ATL 227446 on the ability of growth hormone to bind to liver cell membranes of treated mice, groups of ten mice were treated with every second day for a total of three doses with saline, or with ATL 227446 at 30 mg/kg or with CoC at 30 mg/kg, equivalent to the lowest of the effective ASO doses determined in Experiment 1. Drugs were administered s.c. to the nape of the neck in a volume of 200 µl. At day seven, the animals were killed and the liver tissues removed and processed to determine the relative amount of growth hormone binding to the receptor in isolated total cell membranes.

Experiment 3 A further seven-day study was conducted to determine the effect of ATL 227446 on serum IGF-I levels. Groups of ten mice received three doses of ATL 227446 at either 3 mg/kg or 30 mg/kg, or CoC delivered at 30 mg/kg, or with saline (vehicle control). Drugs were administered s.c. to the nape of the neck in a volume of 200 µl. Blood samples were collected prior to commencement of the study, and at day seven by cardiac puncture (terminal bleed) for assay of serum IGF-I levels.

Experiment 4 To investigate the ability of ATL 227446 to act as a maintenance therapy for IGF-I suppression, a ten-week dosing study was conducted. Groups of 16 mice were treated three times per week for ten weeks with ATL 227446 or CoD at 30 mg/kg, or saline (vehicle control). Doses were administered i.p. in a volume of 200 µl. Over the ten weeks of dosing, and for ten days beyond completion of dosing at day 68, blood samples were collected at intervals for serum IGF-I assessment, and body
weights were regularly measured. To allow for multiple blood sampling, half of each treated group were alternately bled at any single time point so that each mouse was blood sampled only once every two weeks in accordance with ethical requirements. Compounds were delivered on Monday, Wednesday and Friday of each week, with blood sampling occurring on the following Monday prior to delivery of the next set of three doses.

**RNA isolation**

Isolation of total RNA was performed with RNeasy 96-column plates in conjunction with the Qiagen BioRobot 3000 using the manufacturer’s recommended procedures. In brief, one equivalent of 70% ethanol was added to the cell lysis to promote binding of the RNA to the column membranes. Remaining protein and lipid were removed with Qiagen’s proprietary buffers (RW1 and RPE). A solution containing DNase I (Invitrogen Corp.) was then applied to the column membranes (29 units/sample for 15 min) to remove residual genomic DNA. Total RNA was eluted and collected from columns using RNase-free water (Qiagen).

**RNA quantification**

GHR mRNA levels were determined by real-time RT-PCR using the ABI PRISM 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. PCR reagents were obtained from InVitrogen Corporation. RT-PCR reactions were carried out by adding 20 µl PCR cocktail (2·5 × PCR buffer minus MgCl₂, 6·6 mM MgCl₂, 375 µM each of dATP, dCTP, dCTP and dGTP, 375 nM each of forward primer and reverse primer, 125 nM probe, 4 Units RNAse inhibitor, 1·25 Units Taq, 40 cycles of a two-step PCR protocol were carried out by incubation for 30 min at 48 °C to activate the PLATINUM Taq, 5 Units MuLV reverse transcriptase, and 2·5 × ROX dye) to 96-well plates containing 30 µl total RNA solution (20–200 ng). The RT reaction was carried out by incubation for 30 min at 48 °C. Following a 10-min incubation at 95 °C to activate the PLATINUM Taq, 40 cycles of a two-step PCR protocol were carried out: 95 °C for 15 s (denaturation) followed by 60 °C for 1·5 min (annealing/extension). The primers and dual-labelled TaqMan probe utilised for RT-PCR analyses of the mouse growth hormone receptor mRNA were designed from published sequence information (GenBank Acc. No. NM_010284) as follows: forward primer, 5’ TTGACGAAATAGTGGCAACCTGATC 3’; reverse primer, 5’ CGAATCCC-GGTCAAACCTATG 3’; probe, 5’ 6 FAM-CATTGGCCTCAACCTGGACTTTA CT-AA-TAMRA 3’. Data were normalised to the level of 18S rRNA detected. This method was also used for detection of GHR mRNA in mouse liver tissues.

**Assay of serum IGF-I**

Prior to commencement of each study and again at the conclusion of the studies, blood samples (150 µl) were collected by cardiac puncture using a 29-gauge needle, and the serum was separated. Serum IGF-I levels were determined using a commercially available radioimmunoassay kit with acid-ethanol extraction (BioClone, Murrickville, Sydney, NSW, Australia) in accordance with the manufacturers’ instructions. Appropriate inter- and intra-assay controls were used as required. The minimum level of detection of this assay was 45 ng/ml.

**Growth hormone binding assay**

Specific binding of GHR to liver cells isolated from treated mice was assessed with hepatic membranes using a radioligand binding assay (Waters & Friesen 1979). Microsomal membrane preparations were obtained as follows. Tissue powder (400 mg, prepared by grinding liver tissue in liquid nitrogen with a mortar and pestle) was homogenised in cold homogenising buffer (50 mM Tris/HCl, 250 mM sucrose, pH 7·4). This was centrifuged at 300 g for 10 min at 3 °C and the supernatant was saved. This supernatant was centrifuged at 15 100 g for 20 min. Pellets were resuspended in 0·5 ml RRA buffer with inhibitor (50 mM Tris, 20 mM MgCl₂, pH 7·4). The protein content of the microsomal samples was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). Microsomal preparation samples (that include plasma, nuclear and perinuclear membranes) were stored at −80 °C until used in the specific binding assay.

For the 125I-labelled human (h) GH specific binding assay, four glass tubes were prepared for each sample with two replicate positive tubes and two replicate negative tubes. Samples and test reagents were added to each tube as follows: (i) 0·2 ml RRA buffer with 0·1%BSA; (ii) 0·1 ml microsomal preparation at 1·0 mg/ml; (iii) 0·1 ml bovine GH (10 µg/ml) for the positive tubes or (iv) 0·1 ml RRA buffer for the negative tubes; and (v) 0·1 ml 125I-hGH tracer, having a specific activity of 110 µCi/µg and produced by iodogen labelling followed by Sephadex G100 separation.

Samples were incubated at 4 °C with shaking overnight. The reaction was stopped with 2·5 ml cold RRA, and the sample was centrifuged at 1500 g for 25 min at 4 °C. The supernatants were aspirated and the pellets were counted using a γ-counter; specific binding capacity was calculated as a percentage of added counts.

**Statistical analysis**

ANOVA and Bonferroni’s tests or Student’s t-test were used to compare groups or pairs of data sets. Statistics were
calculated using GraphPad Prism statistical software (El Camina Real, San Diego, CA, USA).

Results

Selection of optimised ASO GHR inhibitor using in vitro mRNA knockdown

Seventy-two oligonucleotides rationally designed to target the mouse GHR mRNA were screened in mouse b.END-3 cells (data not shown) to identify the optimal lead compound, ATL 227446, for further in vivo studies. Figure 1 shows the target GHR mRNA levels in b.END-3 cells transfected with ATL 227446 at varying concentrations, in comparison with two control oligonucleotides, CoA and CoB. A distinct dose–response relationship is evident in cells treated with ATL 227446, with up to an 87% knockdown observed when applied at a concentration of 100 nM, and an observed IC₅₀ of ~25 nM. No significant target knockdown was observed for either of the non-target specific control oligonucleotides.

The sequence of the optimal GHR ASO, ATL 227446, is shown below. The ASO is phosphorothioate throughout with 2′-MOE modifications at the five extreme bases of the 5′ and 3′ ends, as indicated by the underlines below. This ASO is directed to the translation initiation codon and is designed to interact with all variants of mouse GHR mRNA. This ASO was used for all subsequent in vivo studies.

GHR ASO, ATL 227446: ACAAAGATCCATACC TGAGA

Treatment of normal mice with ASO directed to mouse GHR reduces GHR mRNA levels in liver tissue

The results of a study to determine the in vivo effects of ATL 227446 on GHR mRNA expression levels are presented in Fig. 2. This shows the levels of GHR mRNA detected in the liver tissue, relative to the saline control group, post-treatment with ATL 227446 versus CoC after one week of treatment. For the 30 mg/kg ASO group, a level of 48% (P<0·0001) knockdown was achieved, with a further decrease being observed following treatment with the higher 50 mg/kg ASO dose (70% reduction, P<0·0001). The mismatch control also induced a mild, but significant (P=0·04) decrease in GHR mRNA, indicating that there was a partial effect of the oligonucleotide alone; however there was a further significant reduction between the control and both the 30 mg (P=0·0001) and the 50 mg (P<0·0001) antisense-treated groups, indicating a GHR ASO specific activity.

Treatment of normal mice with ASO directed to mouse GHR inhibits binding of GH to liver cells (ex-vivo)

Given confirmation of GHR mRNA knockdown by ATL 227446 in vivo, further analysis was conducted to assess the effects of mRNA reduction on target protein expression and activity. Levels of GH binding determined in this study are presented in Fig. 3 and indicate that ASO treatment suppressed the level of GH binding to liver membranes. A 34% (P=0·0017) reduction in binding compared with saline-treated mice was observed with no significant reduction occurring in liver taken from CoC-treated mice.

GHR ASO treatment induces reduction in serum IGF-I levels

As a validated indicator of the level of GH activity in vivo, serum IGF-I levels were assessed in treated mice. Figure 4 shows day seven serum IGF-I levels observed after dosing every second day for seven days with ASO at 3 mg/kg or 30 mg/kg, or with saline, or with CoC administered
at 30 mg/kg. After seven days of treatment of these three-week-old mice, there was a 44% \( (P=0.0017) \) decrease in serum IGF-I values in the ASO 30 mg/kg group relative to the saline-treated group. ASO delivered at 3 mg/kg did not reduce serum IGF-I levels, and while CoC had some effect in target reduction, the level of reduction was not statistically significant. These studies confirm that treatment with GHR ASO induced the expected effects on serum IGF-I levels during growth after as little as seven days of treatment in the higher dose group.

Repeated administration of GHR ASO maintains decreased levels of serum IGF-I over ten weeks of treatment and reduces body weight gain

To investigate the use of GHR ASO as a maintenance therapy for serum IGF-I suppression, groups of mice were treated three times per week for ten weeks with saline, or GHR ASO at 30 mg/kg or CoD control oligonucleotide at 30 mg/kg. Table 1 shows mean serum IGF-I levels recorded in each group at intervals throughout the study. The percentage decrease in serum IGF-I levels relative to the saline control group are shown for the ASO- and CoD-treated groups.

The saline groups exhibited an overall decrease in serum IGF-I levels throughout the course of the study, consistent with the increasing age of the mice. In mice treated with the CoD control oligonucleotide, there was a reduction in serum IGF-I levels relative to saline across the course of the trial, with this difference being statistically significant at the two earliest time points. This result indicates that the oligonucleotide treatment itself exerted some IGF-I reducing activity independent of sequence specificity for GHR. The GHR ASO-treated groups, however, induced a further decrease in serum IGF-I, with a statistically significant decrease relative to both CoD- \( (P<0.02, \text{ data not shown}) \) and saline-treated mice at 30 mg/kg. Table 1 shows mean serum IGF-I levels.
shown, (ASO) or control oligonucleotide (CoD). Group means ± s.e.m.s are shown, (n = 8).

Table 1 at all time points including the sample collected ten days after cessation of dosing (day 78). This confirms the sequence-specific effect of the GHR ASO in the reduction of serum IGF-I levels.

The results of the effects of treatment on mouse body weight over the course of the study are shown in Fig. 5. Initially, the growth curves for the three groups of mice were equivalent, but they begin to diverge after six weeks of treatment. By treatment day 43, the ASO-treated mice had a statistically significant reduction in total body weight compared with both CoD- and saline-treated mice. ASO-induced reduction in body weight was evident for the remainder of the study with the body weight of the ASO-treated mice being 8% lower than the weight of the saline-treated mice at the conclusion of the ten weeks of dosing.

Discussion

There has been considerable development in antisense drug technology in recent years, culminating in successful clinical testing of the first generation, phosphorothioate modified oligonucleotides and those with more advanced chemistries, such as the 2′-MOE modification, as described in this report. Incorporation of the 2′-MOE chemistry to the outer ‘wings’ of an antisense oligonucleotide has resulted in improved hybridisation affinity and stability in vivo, with elimination half lives in the order of thirty days in mice, rats and monkeys (Zhang et al. 1995, Geary et al. 2001a). This has translated into improved efficacy, with up to tenfold lower doses of 2′-MOE modified phosphorothioate compounds being required to achieve the same in vivo effects as the ‘unmodified’ phosphorothioate compounds (Crooke 1998). A range of delivery options, including oral delivery, is now potentially feasible using these more stable compounds (Geary et al. 2001b), which also exhibit enhanced safety due to a reduction in the proinflammatory activities that have been associated with first generation chemistries (Henry et al. 2000). The central 2′ deoxy sugar residues (gap) in the compound are retained to allow the oligonucleotide to act as a substrate for RNase H, the primary mechanism by which the majority of antisense oligonucleotide drugs function (Crooke 1998). The antisense oligonucleotide described in this report is complementary to the AUG start site region of the target transcript, so the additional potential for a translation arrest mechanism cannot be ruled out.

While in vitro analysis as described here is used for selection of optimised antisense sequences, in vivo studies have little predictive value in the selection of appropriate in vivo dosages. In vitro use of ASOs requires transfection of the cultured cells, whereas in vivo delivery is effectively achieved in the absence of any lipid transfection reagents, although the mechanism of uptake is poorly understood (Zhang et al. 2000, Crooke et al. 2005). In vivo studies may be used, however, to examine specificity of the ASO for the target. Doses for the current study were first chosen at the upper end of the range of doses that had previously been successfully used in vivo.

Antisense compounds may offer advantages in the clinic in terms of lack of immunogenicity, dosing frequency or cost, in comparison with protein-based drugs. The most effective use of these drugs is directed by their in vivo tissue distribution, with the liver, kidney, spleen and adipose tissue having high tissue concentrations of antisense oligonucleotides following systemic delivery (Butler et al. 1997). While extrahepatic GH receptors may also be targeted with the ASO described here, the high levels of hepatic accumulation of the drug suggest that its main effects will be on hepatic GH receptors. There may also be some effect in adipose tissues, which bear growth hormone receptors, and to which antisense drugs also distribute. Pharmacokinetic studies have not yet been undertaken for the ATL 227446 ASO; however, there are many published studies describing the disposition of similar antisense oligonucleotides in vivo (Butler et al. 1997, Geary et al. 2001b, c). Using a 2′-MOE ASO, Geary et al. (2003) demonstrated complete, but relatively slow absorption of the drug following subcutaneous delivery, with Tₘₐₓ at approximately 2–3 h. It should be noted that the clinical activity of ASOs is more closely related to the concentration of drugs in relevant tissues rather than in the blood, a compartment from which they are rapidly cleared.

The effect of modulating growth hormone signalling may be measured directly by assessment of the clinically relevant parameter of serum IGF-I concentration, which is secreted by these tissues in response to GH stimulation.
Normalisation of serum IGF-I is the clinically defined cure for acromegaly, a disorder resulting from hypersecretion of GH from a pituitary tumour. Increased levels of serum IGF-I have also been implicated in neovascularisation of the eye (Higgins et al. 2002), which results in diabetic retinopathy and age-related macular degeneration.

The antisense oligonucleotide used for targeting the growth hormone receptor in this study was selected based on its ability to knockdown the target mRNA in vitro. While this assay effectively demonstrated a dose-dependent and significant target knockdown, it was necessary to show that the compound was able to exert this antisense activity in vivo. Assay of GHR mRNA in the livers collected from ASO-treated mice confirmed that there was a dose-dependent and significant decrease after seven days of treatment. Antisense drugs typically have a slower onset of action than conventional drugs due to the need for existing protein to be degraded before the effect of ASO-mediated reduction in target mRNA levels on synthesis of new protein is evident. The time of onset is therefore dependent on the half-life of the target protein, but at least two to three days is generally required to show an antisense effect at the protein level in vivo (Crooke 2004). Complete reductions in mRNA levels may be prevented by a variety of in vivo factors including expression levels and drug access to the target mRNA. As a further demonstration of in vivo efficacy of the GHR antisense compound, there was a significant reduction in the ability of growth hormone to bind liver cell membranes collected from treated mice. The mRNA knockdown had, therefore, translated into a biological effect at the protein level within the first week of treatment.

A slight mismatch effect on both GHR mRNA and serum IGF-I levels occurred, indicating a beneficial and general oligonucleotide effect; however this was separable from the target-specific effect of the GHR ASO. Mismatch effects, either beneficial or detrimental, do occur in a wide range of disease indications, but should be viewed with caution as each new mismatch represents a new molecule which may have its own peculiar effects. The use of multiple oligonucleotide controls serves to clarify the extent of the generalised oligonucleotide effect.

The effect of the antisense compound on serum IGF-I represented the most important parameter in these studies, as it is a clinically relevant endpoint. In mice of this age (approximately three weeks) serum IGF-I levels rise rapidly, and these studies therefore represent a difficult model in which to control IGF-I. Treatment of mice with the GHR antisense compound did, however, have a significant effect in reducing serum IGF-I. After short-term dosing of male mice (seven days), the maximum level of reduction in serum IGF-I levels relative to the untreated control animals was 44%. This reduction is similar to results achieved using pegvisomant (Somavert) after a seven-day treatment schedule in normal female mice (van Neck et al. 2000). This product is currently used in humans for treatment of acromegaly, where decreases in serum IGF-I of 30–70% may be required for normalisation (Trainer et al. 2000). In retinopathy mouse models, reducing serum IGF-I levels by 33% resulted in a 30–43% reduction in retinal neovascularisation (Smith et al. 1997). In humans, reductions in serum IGF-I of ~50% induced by dosing with octreotide resulted in reduced ocular disease and it limited the need for laser therapies (Grant et al. 2000).

The longer ten-week dosing study described here induced up to 59% suppression of IGF-I in ASO-treated mice relative to saline controls. This enhanced suppression relative to the short-term studies may be related to the increased age of the mice at the time of testing and/or to the longer duration of the dosing. The results of the mouse studies described here are therefore a good indication that clinically relevant levels of target knockdown may be achieved in humans following antisense treatment.

The effect of inhibition of GH signalling by the GHR ASO was further demonstrated by the reduced body weight in mice treated with the ASO, relative to saline-treated control mice and mice treated with a control oligonucleotide. This effect on the gain in body weight observed in the GHR ASO-treated mice extended until the conclusion of the study at day 78, ten days after the final dose of ASO was given. While IGF-I mediates many of the indirect effects of growth hormone activity, recent studies have indicated that IGF-I has limited somatogenic action. A direct effect of inhibition of GH activity independent of IGF-I secreted from the liver cannot be ruled out (Sjogren et al. 1999). Other possible causes for weight change such as GH-induced feeding changes have not yet been assessed; however the lack of weight gain change in the mismatch control-treated mice suggests that the effect is specific to the GHR ASO.

In conclusion, our results demonstrate that systemically delivered antisense inhibitors of GHR provide a potentially viable approach to the therapeutic modulation of the GH/IGF-I axis.

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References


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