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Research Report

Human Growth Hormone Increases *SMN* Expression and Survival in Severe Spinal Muscular Atrophy Mouse Model

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Abstract.
Background: Autosomal recessive spinal muscle atrophy (SMA) is characterized by the loss of α motor neurons resulting in progressive muscle loss and respiratory failure. SMA is one of the most common inherited causes of infant death with a carrier frequency of 1 in 50 and a calculated prevalence of about 1 in 11,000 live births in the US. The low amount of functional survival motor neuron (SMN) protein due to mutations or deletion in the *SMN1* gene causes SMA.
Objective: A potential treatment strategy for SMA is to upregulate levels of SMN protein originating from the paralog *SMN2* gene compensating in part for the absence of the *SMN1* gene. Our group has previously shown that activation of the STAT5 pathway by lactation hormone prolactin (PRL) increased SMN levels, improved motor function and enhanced survival in a severe SMA mouse model. Given that human growth hormone (HGH) is also known to activate the STAT5 signalling pathway and is already used extensively in clinical settings, we thus elected to assess its impact on SMN levels.
Methods and Results: Administration of HGH in NT2 cells activated STAT5 pathway which resulted into significant induction in SMN protein levels. Furthermore, systemic administration of HGH to transgenic SMA mice induced SMN protein levels in the brain and spinal cord samples. Critically, HGH treatment improved disease phenotype and increased survival in two severe SMA mouse models.
Conclusions: Our results confirm earlier work suggesting STAT5 pathway activators as potential therapeutic compounds for the treatment of SMA and identify HGH as one such promising agent.

Keywords: Spinal muscular atrophy, human growth hormone, SMN protein, STAT5 pathway, SMA mouse model, therapeutics

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease with a carrier frequency of 1:50 in the US with a calculated prevalence (based on the carrier frequency) of about 1:11000 [1]. Presently, there is no effective treatment for SMA, and it remains as one of the most common genetic causes of infant mortality. The major pathological feature of SMA is the loss of α motor neurons from the anterior horn of spinal cord. The loss of neuromuscular junctions followed by axonal degeneration results in progressive muscle loss and respiratory failure [2]. SMA is caused by low levels of survival of motor neuron (SMN) protein due to deletions of or mutations in the *SMN1* gene [3]. SMN is a 294 amino acid, ubiquitously expressed protein; complete loss of the protein following genetic ablation is embryonically lethal in mice [4]. Due to an evolutionarily recent duplication event, humans uniquely have a paralogous *SMN2* gene which produces a limited amount of full length SMN

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mRNA (∼10%) [5, 6]. All SMA patients have 1 or more copies of *SMN2* gene, which partially compensates for the loss of the *SMN1* gene. Generally, there is an inverse correlation between disease severity and *SMN2* copy number; the greater the number of *SMN2* gene copies, the milder the disease [7, 8]. Since *SMN2* acts as a genetic modifier of the disease, it serves as a SMA therapeutic target [9, 10] with efforts directed at increasing SMN protein from *SMN2* by induction in gene expression, splicing modulation, stabilization of full length functional SMN mRNA [11–16] or protein [17].

We have previously documented an *in vitro* and *in vivo* increase in the full length SMN mRNA and protein following treatment with the lactation hormone prolactin (PRL) through activation of the STAT5 pathway. Treatment of SMA mice with PRL attenuates disease phenotype by reducing weight loss, improving motor function and leading up to 70% extension in survival. The greatest PRL-mediated upregulation in SMN mRNA and protein was observed in mice transgenic for *SMN2*, a larger increase than seen with any other drug to date; indeed the *in vivo* SMN protein levels following PRL induction were comparable to the SMN levels observed in asymptomatic *Smn*+/− mice [13]. Interestingly and unexpectedly (given the relatively recent timing of the genomic duplication event giving rise to *SMN2*), STAT5a transcription binding motifs are seen uniquely in *SMN2* (and not murine SMN or human *SMN1*) promoters. Provided that SMA patients have *SMN2* as the sole source of SMN protein, STAT5a activators may be particularly advantageous for SMA therapy. Although PRL has proven to be a safe and effective treatment for lactation insufficiency [18], the absence of clinical grade PRL poses a major obstacle for further assessment and its utilization as a potential SMA therapeutic. A search of literature revealed human growth hormone (HGH) as a blood brain barrier (BBB) penetrant STAT5 activator with a good clinical safety profile [8, 19, 20]. Thus, we thus elected to test HGH as a potential SMA therapeutic.

We show here that HGH bestowed an increase in SMN protein levels in a human neuronal cell line. Importantly, we show that treatment with HGH increased SMN protein levels in the central nervous system (CNS) tissues of SMA mouse models. In addition, it moderated disease severity and increased survival in two SMA mouse models. Our results provide further evidence that STAT5 pathway activators may serve as potential therapeutic compounds for the treatment of SMA.

MATERIALS AND METHODS

Reagents

HGH was purchased from Eli Lilly and Company (Canada). STAT5 siRNA was purchased from Cell signalling. The antibodies used in this study were SMN/Smn (BD Transduction Laboratories), Actin (Abcam), Tubulin (Abcam), Phospho-STAT5 (Cell signalling) and Total STAT5 (Cell signalling).

Cell culture and drug treatment conditions

Human neuron-committed teratocarcinoma (NT2) cells were maintained in standard conditions (37°C in a 5% CO_2 humidified atmosphere) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1% antibiotics (100 units/ml penicillin-streptomycin) and 2 mM glutamate. NT2 cells were seeded in 6 well plates (5×10^5cells/well) and treated next day with HGH for 24 h.

For the time-course experiment, NT2 cells were seeded in 6 well plates (5×10^5cells/well) and treated beginning 24 hours later with HGH (125 ng/ml) for up to 20 mins.

For siRNA transfections, NT2 cells were seeded in 6 well plates (5×10^5cells/well) and reverse transfected in serum-free DMEM with STAT5 siRNA (50 nM) or nonsilencing control siRNA (50 nM), using RNAiMAX transfection reagent for 48 hours.

ANIMALS

All experiments were carried out in accordance with the Canadian Institute of Health Research (CIHR) Guidebook. All protocols were approved by the University of Ottawa Animal Care and Veterinary Services (ACVS) and Ethics board. The original breeding pair of heterozygous SMNΔ7 mice (*mSmn*+/−, *hSMN2*+/+, *hSMNΔ7*+/+; stock# 005025), Taiwanese mice (*Smn1*tm1Hung Tg(*SMN2*)2Hung/J; stock# 005058) and heterozygous *Smn* knock-out mice (*Smn*+/–) on the FVB background were provided by the Jackson Laboratory. The animals were maintained in an air-conditioned ventilated animal facility. The three SMA mouse models used in this study are all null for murine SMN, (*mSmn*−/−) and are as follows:

1. Taiwanese mice (4 copies of *SMN2*; mild phenotype) [21].
2. SMNΔ7 mice (2 copies of *SMN2*, 2 copies of SMNΔ7; severe phenotype) [22].

3. Taiw/Jax mice (Cross between Taiwanese mice and heterozygous *Smn*+/− mice; 2 copies of *SMN2*; severe phenotype) [16].

Genotyping

Genotyping was performed as previously described by Farooq et al. for SMNΔ7 mice [13]. Genotyping for Taiwanese and Taiw/Jax SMA mice was performed as previously described by Riessland et al. [16] using recommended primers.

Treatment and survival analysis

HGH was diluted in PBS and administered through intraperitoneal (IP) injection using a 30-gauge needle. The pups were randomly assigned to receive HGH or Saline. The experimenters were not blinded as we were required by University of Ottawa ACVS to reveal specific treatments given to each pup on the cage cards. To minimize inter-litter variability, litters were matched with respect to size wherever possible. A two sample *t* test power calculation (alpha = 0.05, power = 0.8 and effect size = 3 days) was used suggesting 4 mice were required per treatment group for survival analysis. Survival, righting time and weight of the pups were monitored daily as described by Avila et al. [15]. A total of 13 SMNΔ7 litters (5 for HGH and 8 for Saline treatment) and 8 Taiw/Jax litters (3 for HGH and 5 for Saline treatment) were used for survival analysis. SMA mice were genotyped at P0 (day of birth) and HGH treatment was started from P1. SMNΔ7 pups who died before P5 were excluded from the study. Mice treatment along with weight and righting time recording were done by 4 different lab members.

Western blot

Cells were washed 2 times with 2 ml PBS (1X) and lysed in 150 µl RIPA buffer containing 10 mg/ml each of aprotinin, PMSF and leupeptin (all from Sigma) for 40 min at 4°C, followed by centrifugation at 13,000 × rpm for 40 min; supernatant was then collected and frozen at −20°C. Animals were sacrificed within 2 hours of final HGH dose (P6) and mice tissues were harvested for Western blot analysis. Samples were homogenized in 0.5 ml RIPA (10 mg/ml each of aprotinin, PMSF and leupeptin). Total protein concentrations were determined by Bradford protein assay using a Bio-Rad protein assay kit. For Western blot analysis, protein samples were separated by 11% SDS-PAGE. Proteins were subsequently transferred onto a nitrocellulose membrane (80 V for 2 h) and incubated in blocking solution (PBS, 5% non-fat milk, 0.05% Tween-20) for 1 h at room temperature followed by overnight incubation with primary antibody at 4°C at the dilution prescribed by the manufacturer. Membranes were washed with PBS-T (PBS with 0.05% Tween-20) 3 times followed by incubation with secondary antibody (anti-mouse or rabbit; Cell signalling) for 1 h at room temperature. Antibody complexes were visualized by autoradiography using the ECL Plus, ECL (GE Healthcare) and ECL clarity (BioRad) Western blotting detection systems. Quantifications were performed by scanning the autoradiographs and signal intensities were determined by densitometric analysis using the ImageJ program.

ELISA

Liver IGF1 levels were analysed with a Mouse/Rat IGF-I Quantikine ELISA kit (R&D Systems) as per the protocol provided by the manufacturer.

Statistical methods

GraphPad Prism software package was used for Kaplan–Meier survival analysis. The log-rank test was used and survival curves were considered significantly different at $P < 0.05$. Data in figures (histograms, points on graphs) are mean values with the standard error mean (SEM) shown as error bars. The Student's two-tailed *t* test was used to evaluate statistical differences between samples and was considered significantly different at $P < 0.05$.

RESULTS

HGH treatment upregulates SMN protein in vitro

To investigate the potential impact of HGH on SMN protein *in vitro*, NT2 cells were incubated at different HGH concentrations (0, 50, 125, 250 and 500 ng/ml) for 24 h and then harvested for Western blot analysis. SMN protein levels were significantly increased at all HGH treatment doses (Fig. 1a and b).

Since it has been reported that the STAT5 pathway regulates *SMN* gene expression [13, 23] and given that HGH has been shown to be an activator of this pathway, we confirmed previously published results that HGH treatment increases phospho and total STAT5 levels, thereby activating the STAT5 pathway (Fig. 2a). To further investigate a potential STAT5 role in *SMN* gene regulation in HGH-induced increase

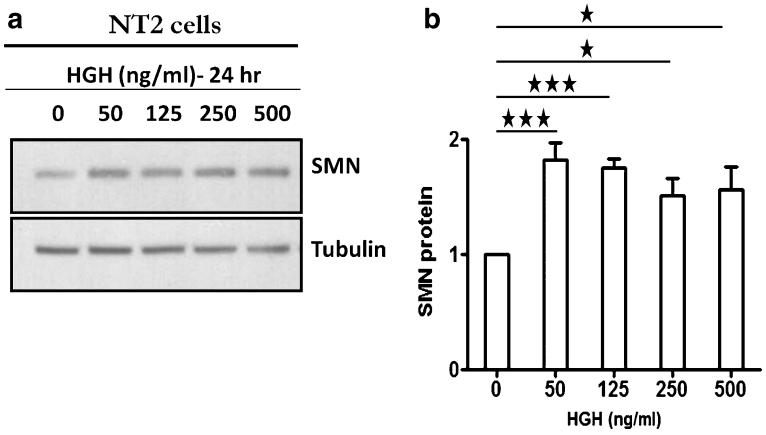

Fig. 1. HGH treatment upregulates SMN protein *in vitro*. NT2 cells were treated with HGH at different doses (0, 50, 125, 250 and 500 ng/ml) and then harvested at 24 hours for Western blot analysis. (a) Representative Western blots showing the effect of HGH treatment on SMN protein in NT2 cells. (b) Densitometric quantification of SMN protein relative to Tubulin [the ratio at control treatment was set as 1; mean + SEM (bars)] of five independent experiments is shown for NT2 cells. $*P<0.05$; $**P<0.01$; $***P<0.001$, *t*-test.

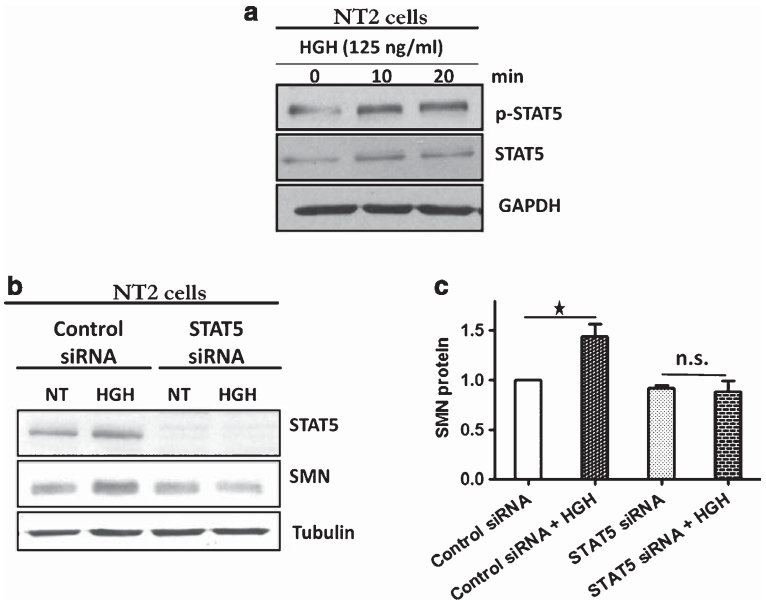

Fig. 2. HGH increases SMN expression via STAT5 pathway. (a) Representative Western blot showing activation of STAT5 pathway upon HGH treatment in NT2 cells. (b) The siRNA knockdown of STAT5 protein attenuates HGH-induced increase in SMN protein. Representative Western blots showing both STAT5 knockdown and its effect on HGH-induced increase in SMN protein. (c) Densitometric quantification of SMN relative to Tubulin [the ratio at control treatment was set as 1; mean + SEM (bars)] of three independent experiments is shown for NT2 cells. n.s. = not significant. $*P<0.05$, *t*-test.

in SMN protein, NT2 cells were reverse transfected with STAT5-specific siRNA or control siRNA for 48 hours and then treated with HGH for 24 hours. siRNA-mediated abrogation of STAT5 expression completely blocked the HGH-induced increase in SMN protein (Fig. 2b and c). These observations strongly implicate the STAT5 pathway in the HGH-induced increase of SMN levels.

HGH treatment upregulates SMN protein levels in vivo

We have previously documented that STAT5 kinase activation by PRL resulted in SMN induction from the human genomic *SMN2* transgene in a murine SMA model [13] and questioned whether similar effect would occur following HGH treatment. To explore this

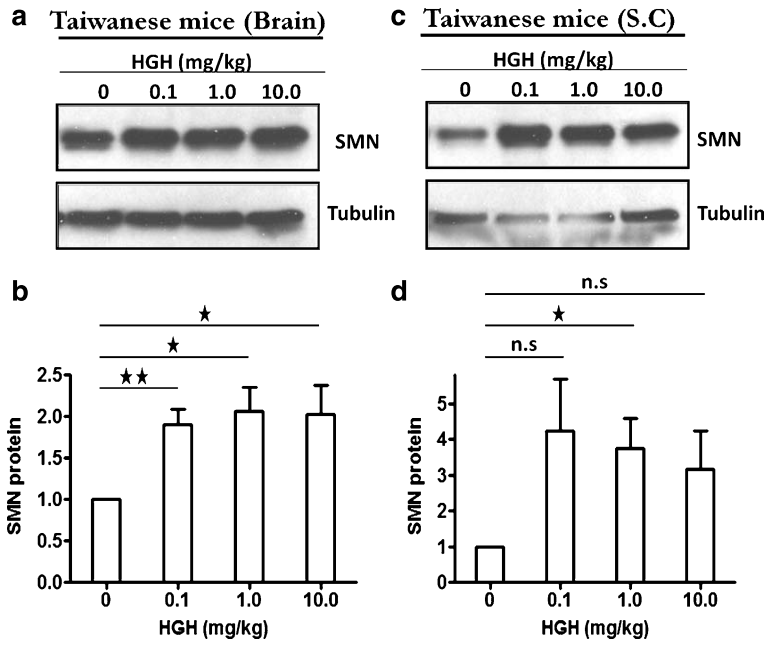

Fig. 3. HGH treatment upregulates SMN protein *in vivo*. 1 week old Taiwanese SMA mice were treated daily with HGH (0, 0.1, 1.0, 10.0 mg/kg) for 5 days, and then sacrificed. Brain and spinal cord tissues were harvested for Western blot analysis. (a) Representative Western blot showing SMN protein levels in brain samples of mice treated with saline (control, lane-1) or HGH (lane 2, 3 & 4) ($n = 3$). (b) Densitometric quantification of SMN relative to Tubulin [mean + SEM (bars)] is shown for brain samples. (c) Representative Western blot showing SMN protein levels in spinal cord samples of mice treated with saline (control, lane-1) or HGH (lane 2, 3 & 4) ($n = 3$). (d) Densitometric quantification of SMN relative to Tubulin [mean + SEM (bars)] is shown for spinal cord samples. n.s. = not significant. $^{*}P < 0.05$; $^{**}P < 0.01$, *t*-test.

possibility, Taiwanese mice (with 4 copies of *SMN2* as the only source of functional SMN protein) were used in order to confirm whether HGH-mediated SMN induction extends to the *in vivo* setting. Mice received daily IP injections of HGH for 5 days at 0.1, 1 and 10 mg/kg. Brain and spinal cord samples were subsequently isolated for Western blot analysis. HGH treatment at all doses increased SMN protein levels two fold or more in brain (Fig. 3a and b). However, in spinal cord samples, a significant increase in SMN protein levels was observed only at 1.0 mg/kg dose (Fig. 3c and d).

HGH treatment upregulates SMN protein levels in a SMNΔ7 mouse model

In order to further explore the *in vivo* impact of HGH conferred SMN protein upregulation, SMNΔ7 SMA mice (*mSmn*−/−;*hSMN2*+/+, *hSMN*Δ7+/+ [22]) were given daily IP injections of 1 mg/kg HGH from P1 until P6. Mice were euthanized 2 hours after their last treatment. Brain, spinal cord, muscle, heart and liver samples were harvested for Western blot analysis. HGH treatment resulted in significant increase in SMN protein levels in both brain (Fig. 4a and b) and spinal cord samples (Fig. 4c and d) compared to saline-treated control animals. No significant induction of SMN protein was observed in skeletal or cardiac muscles of SMA mice following HGH treatment in comparison to saline-treated control mice (Supplementary Figure 1).

A significant increase in SMN protein levels was also observed in liver samples of SMNΔ7 mice following HGH treatment compared to saline treated mice (Supplementary Figure 2a and b). Low levels of insulin-like growth factor 1 (IGF1) has been implicated in mouse SMA pathophysiology [24] and since HGH is a known activator of IGF1, we next examined the IGF1 levels in the liver samples of SMNΔ7 mice. ELISA of liver samples showed a significant increase in IGF1 protein levels following HGH treatment when compared with saline treated mice (Supplementary Figure 2c).

HGH treatment improves disease phenotype in severe SMA mouse models

We next examined the impact of the HGH-mediated increase in SMN levels on the disease phenotype in SMNΔ7 mice. These mice have severely impaired righting times and detectable weakness by P4. They are significantly underweight compared to heterozygous

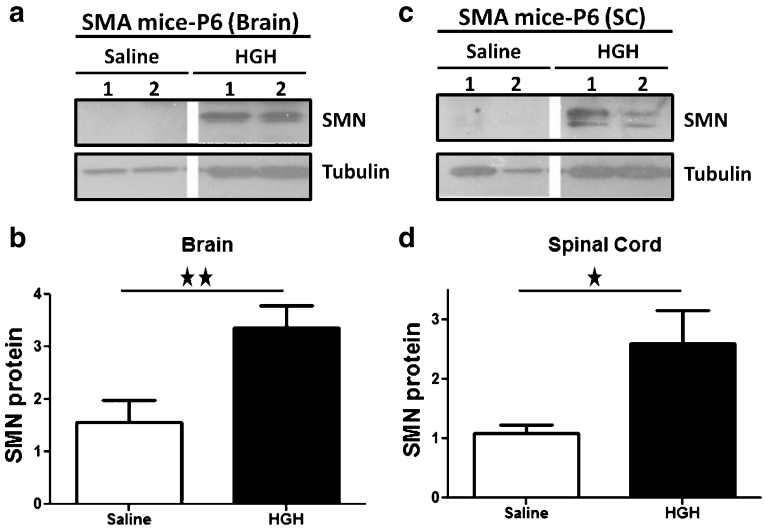

Fig. 4. HGH treatment upregulates SMN protein in SMNΔ7 SMA mouse model. SMNΔ7 mice were treated daily with saline or HGH (1 mg/kg) from P1 for 6 days, then sacrificed at P7. Brain and spinal cord samples were harvested for Western blot analysis. (a) Representative Western blots showing SMN protein in brain samples of SMNΔ7 mice treated with saline (control, lane 1 & 2) or HGH (treatment lane 1 & 2) (each lane represents individual animal; all lanes were run on the same gel but were non-contiguous). (b) Densitometric quantification of SMN relative to Tubulin [mean + SEM (bars)] is shown for brain samples ($n = 10$ for Saline and $n = 9$ for HGH treatment). (c) Representative Western blots showing SMN protein in spinal cord samples of SMNΔ7 mice treated with saline (control, lane 1& 2) or HGH (treatment lane 1 & 2) (each lane represents an individual animal). (d) Densitometric quantification of SMN relative to Tubulin [mean + SEM (bars)] is shown for spinal cord samples ($n = 10$ for saline and $n = 9$ for HGH treatment). $^{*}P < 0.05$; $^{**}P < 0.01$, t-test.

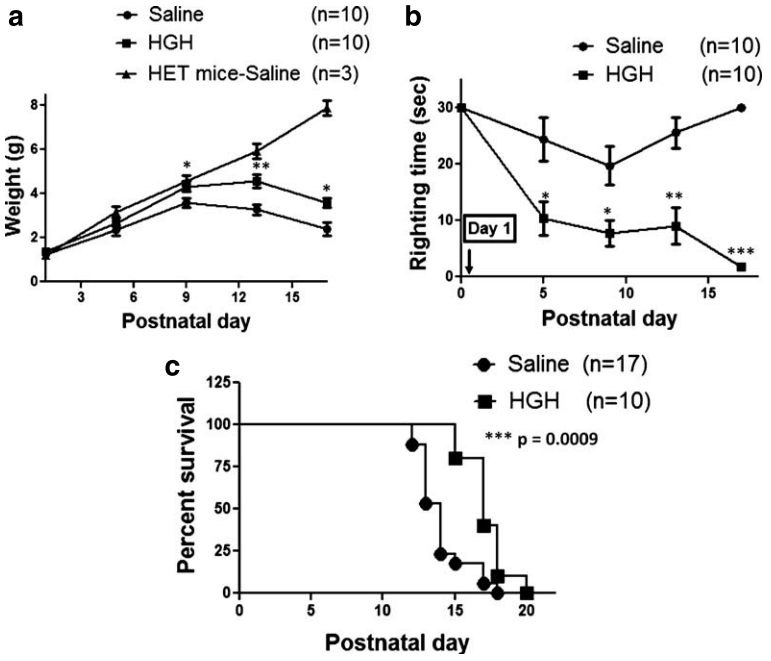

Fig. 5. HGH ameliorates disease phenotype and increases survival of SMNΔ7 SMA mouse model. SMNΔ7 mice were treated daily with IP injections of HGH (1 mg/kg) from P1 onward. (a) Weights of SMNΔ7 mice treated with HGH (black filled square, $n = 10$) or saline (black filled circle, $n = 10$); weights for heterozygous mice treated with saline (black filled triangle, $n = 3$) are also shown for comparison [mean ± SEM (bars)]; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, t-test. (b) Righting times of SMNΔ7 mice treated with HGH (black filled square, $n = 10$) or saline (black filled circle, $n = 10$) [mean ± SEM (bars)]; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, t-test. (c) Kaplan-Meier survival curves of SMNΔ7 mice treated with HGH (black filled square, $n = 10$) or saline (black filled circle, $n = 17$); $^{***}P < 0.001$, log-rank test.

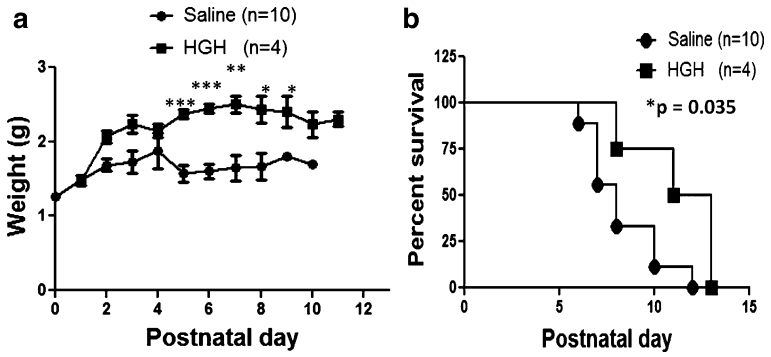

Fig. 6. HGH ameliorates disease phenotype and increases survival of a second severe SMA mouse model. Taiw/Jax SMA mice were treated daily with IP injections of HGH (1 mg/kg) from P1 onward. (a) Weights of Taiw/Jax SMA mice treated with HGH (black filled square, $n = 4$) or saline (black filled circle, $n = 10$) [mean ± SEM (bars)]; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, t-test. (b) Kaplan-Meier survival curves of Taiw/Jax SMA mice treated with HGH (black filled square, $n = 4$) or saline (black filled circle, $n = 10$); $^{*}P < 0.05$, log-rank test.

and wild type (WT) littermates with a median survival of 13 days. SMNΔ7 mice received daily IP injections of 1 mg/kg HGH or saline starting at P1 and their weight, motor function and survival was assessed daily. SMA mice treated with HGH showed improved weight gain (from P5 onwards) and motor function (assessed by righting time; shown in supplementary video), and significant extension of survival (median survival of 17 days) compared to saline-treated SMNΔ7 mice (median survival of 14 days, Fig. 5a, b and c).

We also treated another severe SMA mice model (Taiw/Jax), which are also significantly underweight compared to heterozygous and WT littermates with a median survival of 8 days [16]. Taiw/Jax mice received daily IP injections of HGH (1.0 mg/kg) or saline starting at P1 and their weight and survival were assessed. The HGH-treated mice showed improved weight gain (from P5 onwards) and a significant extension of survival (median survival of 12 days) compared to saline-treated Taiw/Jax mice (median survival of 8 days, Fig. 6a and b).

DISCUSSION

SMA is a deadly neurodegenerative disease and a leading inherited cause of infant death. More than 50% of those born with SMA have type I SMA (the most severe form), and thus usually succumb to the disease prior to five years of age. One potential SMA therapeutic approach is to induce the *SMN1* paralog *SMN2* to produce more SMN protein thereby moderating disease severity. We and others have previously identified the STAT5a pathway as one means of achieving such induction. Ting et al. have reported that treatment with sodium valproate, trichostatin A (TSA) and aclarubicin results in an *in vitro* activation of STAT5a leading to increased *SMN2* expression [23]. Our group has also reported that transcriptional activation of the STAT5 pathway by PRL increases SMN levels *in vitro* as well as in SMA mouse model. PRL also improved the disease phenotype and survival in a severe SMA mouse model [13]. However, the absence of clinical grade PRL has hindered the initiation of PRL clinical trials for SMA. This prompted us to evaluate other safe STAT5 activating compounds as an alternative to PRL for development as a therapeutic for SMA. Since HGH (a compound in wide clinical use) has been reported to cross BBB and activate the STAT5 kinase pathway [8, 19, 20], we elected to assess its potential as an inducer of SMN protein both *in vitro* and *in vivo*.

Here, we report a HGH-mediated increase of SMN protein in human NT2 cells. We also show an increase in both phospho and total STAT5 levels upon HGH treatment. To confirm the role of STAT5 activation in HGH-mediated increase in SMN protein, cells were pre-treated with STAT5 siRNA, which resulted in attenuation of HGH-mediated SMN induction.

In order to confirm that HGH-mediated SMN protein induction extends to the *in vivo* setting, a dose-finding study was initiated. Since the *SMN2* promoter is uniquely responsive to STAT5a activation (in contrast to *SMN1* and mouse *Smn* promoters), a transgenic mild SMA mouse model (Taiwanese mice) with 4 copies of *SMN2* as a sole source of SMN protein, was initially used. We found a significant sustained increase in SMN protein levels in CNS tissues. We next investigated the effect of HGH on SMN protein levels and clinical severity in the SMNΔ7 mouse model. These SMA transgenic mice lack the murine *Smn* gene and have two human transgenic alleles, one of which is human *SMN2* in its genomic chromosomal context [22]; consequently we believed there was a good prospect of

observing *SMN2* induction in this model with HGH treatment. Consistent with this, SMA mice treated with HGH demonstrated a doubling and even tripling in *SMN2*-derived full length SMN protein levels in CNS tissues. Although this induction was significant, it was less than what was observed with PRL treatment. This may be a result of HGH being a more potent activator of STAT5b than STAT5a [25, 26] and *SMN2* promoter uniquely having STAT5a transcription binding motifs. We also examined the impact of HGH on survival in two severe SMA mouse models (SMNΔ7 [22] and Taiw/Jax-SMA [16]). SMNΔ7 mouse closely resembles a severe type II SMA phenotype in humans and show an impaired righting reflex by P4, fail to gain weight and usually succumbs to the disease by 13 days. Taiw/Jax mice also have a severe SMA phenotype, usually living 6–8 days. Treatment of SMNΔ7 mice with HGH resulted in improved weight gain and motor function when compared to saline-treated mice. Taiw/Jax mice also showed improved weight gain compared to control mice. To best gauge the impact of HGH on survival and account for the variability between various laboratories and mouse models, the ratio of median survival of treated to control animals was calculated. With HGH, we have achieved a ratio of 1.21 (17d/14d for SMNΔ7) & 1.5 (12d/8d for Taiw/Jax), which compares favourably with treatment such as TSA (1.2; 19d/16d albeit P5 TSA initiation in SMNΔ7) [15], SAHA (1.3; 12.9d/9.9d in Taiw/Jax-SMA) [16], BAY 55-9837 (1.39; 19.5d/14d for SMNΔ7 and 1.5; 12d/8d in Taiw/Jax-SMA) [13] and celecoxib (1.38; 18d/13d for SMNΔ7) [11]. Prolactin treatment resulted in better survival (1.5; 21d/14d for SMNΔ7) [13] and attenuation of disease phenotype when compared to HGH, possibly a reflection of the greater SMN induction observed with PRL treatment.

SMA treatment strategies have traditionally focused on drugs that can cross the BBB to target tissues within the CNS. However, several recent studies suggest a role for SMN above and beyond motor neurons and challenges SMA's classification as an exclusively motor neuron disease [24, 27–34]. In this regard, we observed no increase in SMN protein in the SMA mice muscle tissues upon HGH treatment. It is worth to note that, unlike the case in the mouse where it has been suggested that cardiac failure is a significant clinical factor, extra-neuronal disease manifestations appear less significant in humans in comparison to denervation which is likely still the chief arbiter of human SMA severity. A significant increase in SMN protein levels was observed in liver samples of SMNΔ7 mice upon HGH treatment. The IGF1 system is disrupted in many neurodegenerative diseases including SMA [24, 35, 36]. Since HGH is a known activator of IGF1 [37], we elected to assess whether hepatic IGF1 levels are changed upon HGH treatment in SMNΔ7 mice. SMA mice treated with HGH showed significant increase in hepatic IGF1 levels. It remains an open question whether the modulation of hepatic IGF1 levels has implications in amelioration of disease phenotype in SMA mice.

Contrary to public perception, HGH does not appear to enhance muscle strength in the normal population [38] nor in individuals with growth hormone deficiency [39–43]. Nonetheless, a single SMA patient reported improvement in muscle strength after long term subcutaneous HGH treatment (0.01 mg/kg) which led to a phase 2 trial to determine whether HGH treatment can safely increase strength in people with SMA type II and III [44]. In this pilot study, SMA type II/III patients showed no improvement in muscle strength or function upon HGH treatment for 3 months [44]. However it should be noted that the dose used in clinical trial (0.03 mg/kg/day) was one thirtieth of that used in this study (1 mg/kg). Furthermore, the general experience has been that early treatment is critical to maximum benefit in mouse SMA models [45, 46], which may well be the case in humans as well. We believe these factors should be taken into consideration before crossing out HGH from the list of potential SMA therapeutics. Particularly, a HGH dose escalation study approximating the doses used here in younger SMA patient population in which lymphocyte SMN levels are assayed should be considered.

Here, we report the first demonstration of HGH-conferred increase in SMN protein level, strength and survival in animal models of SMA. Although the treatment effect is not dramatic, we believe it offers promise for slowing disease progression while providing further supportive evidence for STAT5 kinase pathway activation as a therapeutic approach for SMA.

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SUPPLEMENTARY MATERIAL

Supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JND-140000.

Supplementary Video. SMNΔ7 mice - P13-Saline vs. HGH treatment.

Supplementary Figure 1. (a) Representative Western blots showing SMN protein in skeletal muscle samples of SMNΔ7 mice treated with saline (control, lane 1 & 2) or HGH (treatment lane 1 & 2) (each lane represents individual animal; all lanes were run on the same gel but were non-contiguous). (b) Densitometric quantification of SMN relative to Actin [mean + SEM (bars)] is shown for skeletal muscle samples ($n = 8$ for Saline and $n = 10$ for HGH treatment). (c) Representative Western blots showing SMN protein in heart samples of SMNΔ7 mice treated with saline (control, lane 1 & 2) or HGH (treatment lane 1 & 2; each lane represents an individual animal; all lanes were run on the same gel but were non-contiguous). (d) Densitometric quantification of SMN relative to Tubulin [mean + SEM (bars)] is shown for heart samples ($n = 10$ for saline and $n = 8$ for HGH treatment). n.s. = not significant. $^{*}P < 0.05$; $^{**}P < 0.01$; *t*-test.

Supplementary Figure 2. (a) Representative Western blots showing SMN protein in liver samples of SMNΔ7 mice treated with saline (control, lane 1 & 2) or HGH (treatment lane 1 & 2) (each lane represents individual animal). (b) Densitometric quantification of SMN relative to Actin [mean + SEM (bars)] is shown for liver samples ($n = 5$ for Saline and $n = 5$ for HGH treatment). (c) IGF1 liver levels in SMNΔ7 mice treated with saline or HGH ($n = 5$ for each treatment group) as measured by ELISA. $^{**}P < 0.01$; *t*-test.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to report.

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