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ORGAN TOXICITY AND MECHANISMS

Development of human growth hormone-treated chimeric mice with humanized livers for an evaluation model of drug-induced fatty liver disease

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Abstract

Chimeric mice with humanized livers were used to evaluate drug-induced liver injury (DILI). However, lipid accumulation is observed in the human hepatocytes of chimeric mice because of human growth hormone deficiency (GHD), which is an obstacle in the evaluation of drug-induced fatty liver disease (DIFLD), a common type of DILI. Previously, we showed that lipid droplets were reduced by the administration of human growth hormone (h-GH) to chimeric mice. Although h-GH administration reduces the lipid droplets, an optimal h-GH treatment method for assessing DIFLD has not yet been developed. This study investigated the appropriate h-GH dosage required to reduce lipid droplets and reproduce physiological conditions in humans. Moreover, the LXR agonist TO901317 was administered to h-GH-treated chimeric mice to evaluate the new h-GH treatment's effectiveness for DIFLD assessment. The results in blood h-GH levels, oil-red O liver sections, and gene expression levels in the liver suggested that 0.25 mg/kg/day would be an appropriate h-GH dosage to reduce lipid droplets and reproduce human physiological condition. At this dose, TO901317-induced lipid accumulation and lipid synthesis-related gene expression in humanized livers in a dose-dependent manner, suggesting that this new mouse model could be useful for evaluating human DIFLD. In summary, the administration of h-GH at an appropriate dosage regulated lipid homeostasis in the humanized livers of chimeric mice and h-GH-administered chimeric mice may represent a highly sensitive evaluation model for human DIFLD. The study also suggests a correlation between GH levels and lipid metabolism, potentially related to conditions like GHD and aging.

Keywords Growth hormone · cDNA-uPA/SCID mice · Humanized liver · Drug-induced fatty liver

Introduction

Drug-induced liver injury (DILI) is a common occurrence caused by nearly all classes of drugs. Drug-induced fatty liver disease (DIFLD), a specific form of DILI, is caused by drugs that act via different signaling pathways. Therefore, an in vivo model for evaluating DIFLD would be useful for predicting human DIFLD and analyzing the molecular mechanism of lipidosis in the human liver.

Although many animal models have been utilized in preclinical studies to predict DIFLD in humans, it is necessary to consider species differences in the mechanism of lipid synthesis regulation. A previous report indicated that a 2-week administration of ursodeoxycholic acid, a clinically used bile acid, reduced farnesoid X receptor activity and increased the expression of stearoyl-CoA desaturase (*SCD*), a lipid synthesis gene, and triglycerides (TG) in the human

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liver, whereas ursodeoxycholic acid treatment reduced TG levels in mouse livers (Mueller et al. 2015). This is thought to be due to species differences in uncharacterized mechanisms in the liver.

To study the pathophysiological characteristics of the human liver, we previously developed a humanized liver chimeric mouse by xenotransplanting human hepatocytes into urokinase-type plasminogen cDNA/severe combined immunodeficient (cDNA-uPA/SCID) mice (Tateno et al. 2015). In our previous study, we observed that the engrafted cells maintained their original hepatocyte characteristics in the host mouse liver, and more than 80% of the gene expression levels were comparable (less than twofold) to those in human clinical tissue samples. This chimeric mice have been used as human models to evaluate liver injury by reactive metabolites (Ishida et al. 2020; Matsunaga et al. 2021), mitochondrial toxicity (Kakuni et al. 2012; Yamazaki et al. 2015), phospholipidosis (Sanoh et al. 2017), antibody drugs (Nihira et al. 2019), genotoxicity (Tateno et al. 2019), and nuclear receptor activation (Yamada et al. 2020), suggesting that they could also be useful for evaluating DIFLD.

However, the humanized liver chimeric mouse would not be sufficient as an appropriate animal model for the prediction of DIFLD because this model spontaneously develops fatty liver. Seven weeks after hepatocyte transplantation, lipid accumulation was confirmed by Oil Red O staining (ORO) (Tateno et al. 2011). Although a recent in vitro study showed that the accumulated lipids in human hepatocytes isolated from chimeric mice were reduced by using a 40% oxygen culture system to predict DILI (Ohtsuki et al. 2023), an in vivo assessment system using lipid-reduced humanized liver mice has not yet been developed. It is supposed that the liver steatosis in the humanized liver mouse was caused by limited or no cross-reactivity between mouse growth hormone (m-GH) derived from host mouse pituitary grand and human growth hormone receptor (h-GHR) expressed by engrafted human hepatocytes (Souza et al. 1995; Tateno et al. 2011). Previous studies have shown that patients with GH deficiency (GHD) often have fatty liver or nonalcoholic steatohepatitis comorbidities (Doycheva et al. 2022), and that the knockout of m-GHR also results in fatty liver (Fan et al. 2009). Our group has also shown that 14 days of hGH administration at 2.5 mg/kg/day suppressed the expression of lipid synthesis-related genes, resulting in reduced lipid accumulation in the liver (Tateno et al. 2011). Therefore, h-GH-treated chimeric mice, which show a reduction in lipid droplets, are promising as an evaluation model of DIFLD. However, the blood h-GH levels in chimeric mice have not yet been analyzed following exogenous h-GH treatment. Additionally, this conventional dosage is high compared to clinical conditions because somatropin, an h-GH preparation used to improve short stature and fatty liver in patients with GHD or Turner's syndrome, is injected within a range of approximately 0.021 ~ 0.47 mg/kg/day. Such a high dose of h-GH could also suppress drug-induced fat accumulation in chimeric mouse liver. Therefore, a new GH dosage should be explored to develop an animal model for the evaluation of DIFLD that presents fewer lipid droplets in the liver and reproduces human physiological conditions. Furthermore, although a correlation between GH levels and lipid metabolism has been suggested for several phenomena, including GHD and aging (Doycheva et al. 2022; Sumida et al. 2015), the detailed mechanism remains unclear, and it could potentially be elucidated through studies of h-GH function in chimeric mice.

Thus, in this study, we first optimized the h-GH dosage in humanized liver chimeric mice to improve fatty liver and physiological blood h-GH levels, and to modulate GH-related gene expression levels closer to those in humans. We also administered TO901317, a potent inducer of fatty liver (Chisholm et al. 2003; Grefhorst et al. 2002; Kim et al. 2021), to h-GH-treated chimeric mice to examine whether the h-GH-treated model could develop a fatty liver. In addition, TO901317 is known to activate lipid synthesis-related genes (Chisholm et al. 2003; Grefhorst et al. 2002; Kim et al. 2021), inhibit GH signaling in rodent hepatocytes (Zadjali et al. 2011), activate *ATP binding cassette subfamily A member 1* (*ABCA1*, a target gene of liver X receptor (LXR)) (Tamehiro et al. 2007), and inhibit *carnitine palmitoyl transferase 1A* (*CPT1a*, a fatty acid oxidation-related gene) (Ide et al. 2003). The activation of *ABCA1* induced lipid accumulation and also enhanced lipid excretion, indicating a dual role for LXR in improving cholesterol metabolism and inducing fatty liver (Zhang et al. 2012). However, the relationship between these mechanisms in the human hepatocytes remains unclear. Therefore, we investigated these effects of TO901317 in human hepatocytes in the chimeric mice treated with hGH.

Materials and methods

Animals

Chimeric mice with human hepatocytes were generated as previously described (Tateno et al. 2015). cDNA-uPA hemizygous male mice were used as hosts for all transplantation experiments. Human hepatocytes (Lot; BD195:2-year-old Hispanic female; BD Biosciences, Ann Arbor, MI, USA) were transplanted into 2–4 weeks old cDNA-uPA/SCID mice (1.0×10^6 cells/animal). The occupancy ratio of h-hepatocytes in host mouse livers was estimated using human blood albumin levels as described in our previous report (Tateno et al. 2004, 2015). A total of 31 chimeric mice (estimated occupancy rate 85–98%) were sacrificed—15–25 weeks

after transplantation for the optimization of rhGH (15 mice) and confirmation of drug-induced liver steatosis (16 mice).

Administration of recombinant h-GH

From day 0, 15 chimeric mice were continuously infused with recombinant h-GH (FUJIFILM Wako Chemicals, Osaka, Japan) at 0.1, 0.25, 0.5, 1.0, or 2.5 mg/kg/day through subcutaneous injection (s.c.)-implanted Alzet micro-osmotic pumps (Alza Corp., Palo Alto, CA, USA) for 14 days before sacrificing. Serum h-GH and human insulin-like growth factor 1 (h-IGF1) levels were determined using the human growth hormone Enzyme-linked Immunosorbent Assay (ELISA) Kit (Abcam, Cambridge, UK) and the Human IGF-I/IGF-1 Quantikine ELISA Kit (R&D Systems, Minneapolis, USA), respectively.

Co-administration of TO901317 with h-GH

Sixteen chimeric mice were continuously infused with 0.25 mg/kg/day h-GH through the osmotic pumps for 14 days before sacrificing. From day 10, the mice were simultaneously orally administered vehicle (0.5% w/v Methyl Cellulose 400 Solution, FUJIFILM Wako Chemicals), or 10, 30, or 100 mg/kg/day TO901317 (Cayman Chemical, Michigan, USA) daily for 4 days before sacrifice.

Drug dosages were determined based on previous studies in mice (Chisholm et al. 2003; Grefhorst et al. 2002; Kim et al. 2021). Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and TG levels were quantified using a Fuji DRI-CHEM (Fuji FILM, Tokyo, Japan).

Histochemistry and lipid staining of chimeric mouse liver

Formalin-fixed paraffin-embedded Sects. (5 µm thick) of chimeric mouse livers were prepared for hematoxylin and eosin (H&E) staining. ORO staining was performed as previously described (Kisoh et al. 2021) using frozen liver Sects. (8 µm) and commercially available reagent (Oil Red O solution, Muto pure chemical Co., Ltd., Tokyo, Japan). All images were acquired using a BZ-X710 microscope (Keyence, Osaka, Japan), and the ORO-positive area was quantified using BZ-X analysis software (Keyence).

Gene expression analysis

Total RNA was isolated from chimeric mouse liver tissues using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. One microgram of total RNA was used as a template of cDNA synthesis with Super Script III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and oligo-dT primers (Invitrogen), following the manufacturer's instructions. Quantitative RT-PCR (qRT-PCR) analysis was conducted using SYBR Green PCR master mix (Applied Biosystems, Tokyo, Japan), specific primer pairs, and a 7500 Real-Time PCR System (Applied Biosystems), as previously described (Kisoh et al. 2021). The species specificity of these primers was confirmed using mouse or human liver cDNA. Gene expression levels were normalized to that of *human glyceraldehyde 3-phosphate dehydrogenase (h-GAPDH)*. The primer sequences and annealing temperatures for each gene are listed in Table 1.

Statistics

Data were analyzed using Statcel 4 software (OMS Publishing Inc., Tokorozawa, Japan). Results are expressed as the mean ± standard deviation (SD). Multiple sample comparisons were made using one-way analysis of variance followed by Tukey's post hoc test or Dunnett's T3 test. Statistical significance was considered at p-values < 0.05 or 0.01.

Results

Blood h-GH and h-IGF1 levels

Chimeric mice received implantation of osmotic pumps containing different dose of recombinant h-GH (0.1, 0.25, 0.5, 1, and 2.5 mg/kg/day) for the search of an appropriate dosage. Serum levels of h-GH and h-IGF1, downstream genes of h-GH signaling (Vázquez-Borrego et al. 2023), were analyzed using sandwich ELISA on days 1, 2, 4, 7, and 14. Serum concentrations of h-GH were detected on day 1 and remained constant until day 14 after the continuous infusion of h-GH (Fig. 1A). At 0.25 mg/kg, a dose within the range of somatropin dosage for patients with GHD (approximately 0.021 ~ 0.47 mg/kg/day), these levels were 0.7 ng/mL at day 7 and 2.1 ng/mL at day 14, which were close to those of human adults (approximately 0.1 ~ 5 ng/mL) (Brandenberger and Weibel 2004; Takahashi et al. 1968). The serum concentration levels of h-IGF1 gradually increased throughout the monitoring period (45.8 ng/mL on day 14), and the levels depended on the h-GH dosage (Fig. 1B). Considering that the serum h-IGF1 levels in human adults are 50 ~ 350 ng/mL (Isojima et al. 2012), we concluded that serum concentrations of h-GH and h-IGF1 were maintained at physiological levels by continuous administration of h-GH at 0.25 mg/kg for two weeks.

Hepatic lipid droplets

The effect of h-GH dosing on the liver steatosis was examined using H&E- or ORO-stained liver sections. Mice were autopsied after 14 days of h-GH treatment, and lipid

Table 1 List of primers used in this study

Genes		Sequences (5′→3′)	Annealing temperature (℃)
h-GAPDH	F:	GGAGTCAACGGATTTGGT	60
	R:	AAGATGGTGATGGGATTTCCA	
h-IGF1	F:	ATCAGCAGTCTTCCAACCCA	60
	R:	TGGTGTGCATCTTCACCTTCA	
h-SOCS2	F:	GGTCGGCGGAGGAGCCATCC	60
	R:	GAAAGTTCCTTCTGGTGCCTCTT	
h-GHR	F:	GTATCAAGCTAACTAGCAATGGTG	65
	R:	TTCCCATCTCACTTGGATATCTG	
h-SCD	F:	TCAAAACAGTGTGTTCGTTGC	60
	R:	CATAAGGACGATATCCGAAGAGG	
h-ABCA1	F:	GCCAGAAAGACACCAGCATG	60
	R:	GAGAGAGGTTGTGATACAGGA AC	
h-CPT1a	F:	GACTCTGGAAACGGCCAACT	60
	R:	ATCTTGCCGTGCTCAGTGAA	
h-SREBP1c	F:	CCAGCATAGGGTGGGTCAAA	65
	R:	GACCGACATCGAAGACATGC	
h-FADS1	F:	CAGGCCACATGCAATGTC	65
	R:	ATCTAGCCAGAGCTGCCCTG	
h-FASN	F:	ATTGGCAAATTCGACCTTTCTCAG	60
	R:	GCACTGCTCTCGTTGAAGAAC	

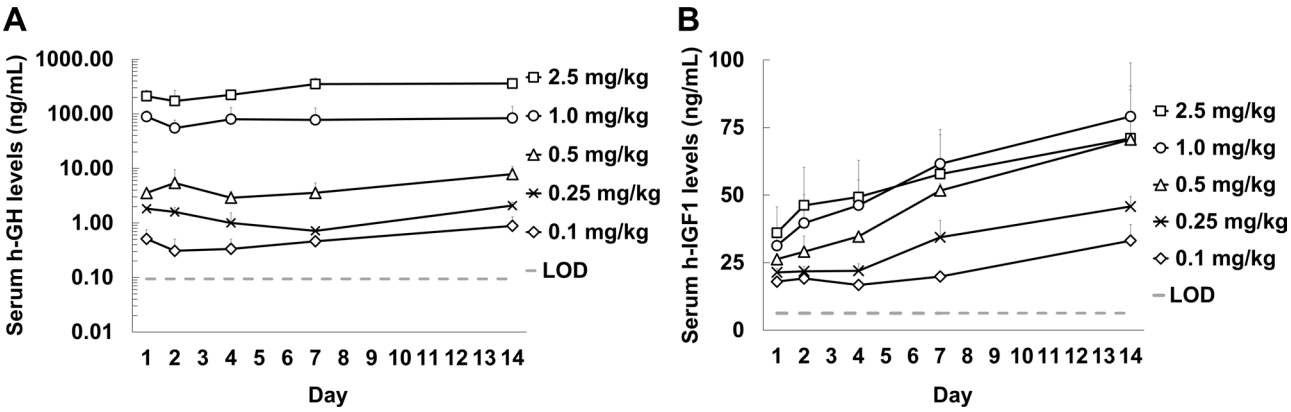

Fig. 1 Serum h-GH and h-IGF1 concentrations in the humanized mice administered with h-GH at different doses ($n=3$ each). Time course changes of serum h-GH (**A**) and h-IGF1 (**B**) concentration levels with each h-GH dosage at day 1, 2, 4, 7, and 14. The dotted lines represent each limit of detection (LOD) (h-GH, 0.094 ng/mL; h-IGF1, 6.4 ng/mL). Data represent the mean ± SD

droplets were observed as whitish or red regions after H&E (Fig. 2A) or ORO staining (Fig. 2B(a)), respectively. Compared to h-GH-untreated mice, 0.25 mg/kg and higher h-GH administration significantly decreased lipid droplets in each stained section (Fig. 2B(b)).

Hepatic gene expression

The mRNA expression of genes related to GH signaling or lipid homeostasis was analyzed using real-time qRT-PCR (Fig. 2C). The mRNA expression levels of *h-IGF1*

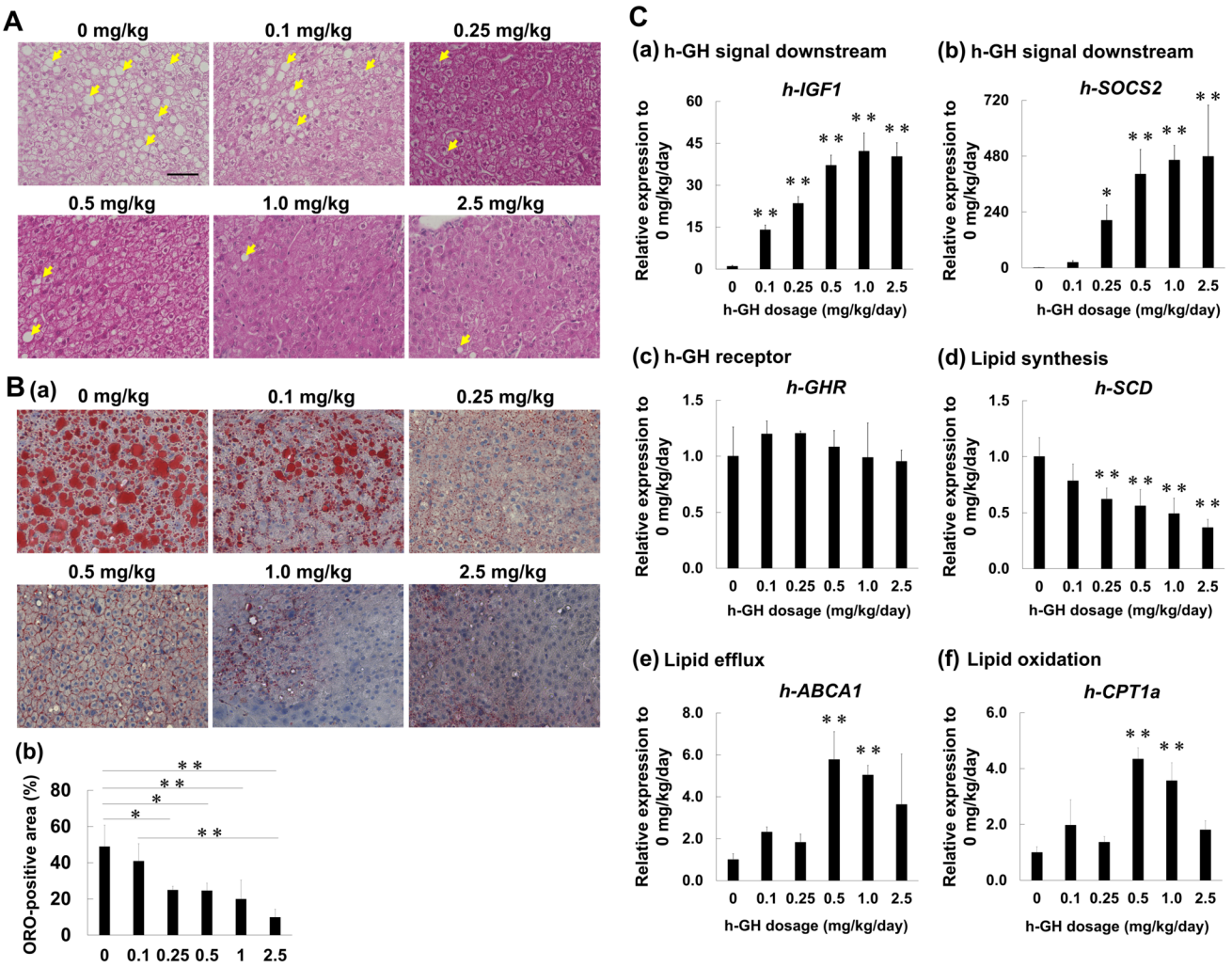

Fig. 2 Effects of h-GH administration on liver histology and gene expression profiles in human hepatocytes of the chimeric mice. **A** Histological examination by H&E staining. Yellow arrows indicate the lipid droplets. **B** Representative images of ORO-stained liver sections (a) and results of quantitative analysis of the ORO-positive area (b). Bar, 100 µm. Asterisks above the lines indicate significance among the groups (*$P<0.05$, **$P<0.01$, Tukey–Kramer test). **C** Results of real-time qRT-PCR for the indicated genes. At day 14, RNA was isolated from the liver tissue for real-time qRT-PCR analysis. Asterisks above each bar indicate significance between the each dosage group and the 0 mg/kg h-GH treated group (*$P<0.05$, **$P<0.01$, Dunnett's T3 test). Data represent the mean ± SD

and *human suppressor of cytokine signaling 2* (*h-SOCS2*), downstream of GH signaling in chimeric mice, were increased by h-GH administration (Fig. 2C(a, b)), whereas the *h-GHR* expression levels did not change (Fig. 2C(c)). The levels of *h-SCD,* lipid synthesis-related gene, were suppressed by h-GH administration at higher than 0.1 mg/kg (Fig. 2C(d)). Additionally, the expression levels of human ABCA1 (*h-ABCA1*) and CPT1a (*h-CPT1a*), genes of lipid metabolism-related transporters and fatty acid oxidation, respectively, were increased at 0.5 and 1.0 mg/kg (Fig. 2C(e, f)). These data suggest that exogenous h-GH activates the GH signaling cascade, reduces lipid synthesis, and affects lipid excretion and beta-oxidation in chimeric mouse liver.

Evaluation of lipid accumulation by TO901317

To examine whether the h-GH-administered humanized liver mouse is a suitable model animal for DIFLD, h-GH-treated chimeric mice were administered TO901317, a potent chemical inducer of fatty liver. From day 10, TO901317 was orally co-administered daily for 4 days (Fig. 3A). The results of ORO staining revealed that TO901317 treatment significantly induced lipid accumulation at this dosing schedule (Fig. 3B).

To analyze the influence of TO901317 treatment on lipogenesis in the humanized liver, the expression of *h-SCD, human sterol regulatory element-binding protein 1c* (*h-SREBP1c*), *human fatty acid desaturase 1* (*h-FADS1*),

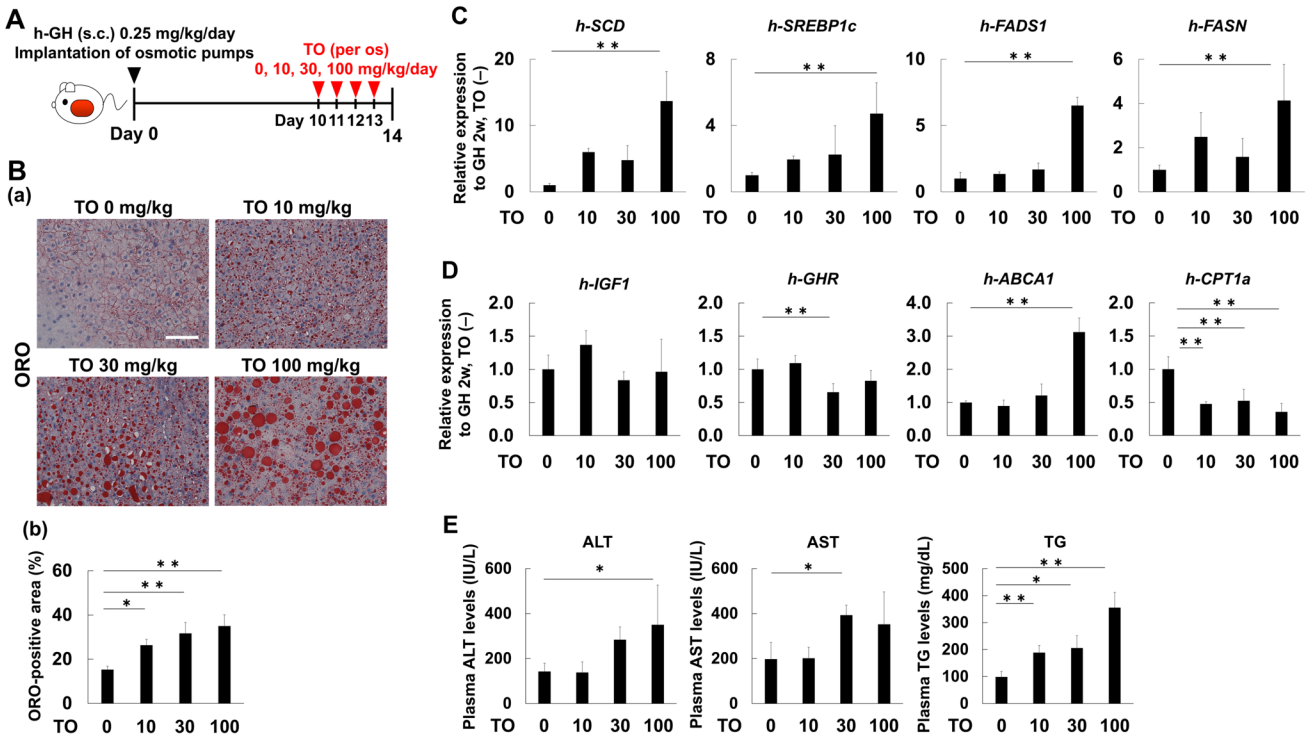

Fig. 3 Induction of lipid accumulation by TO901317 (TO) in the h-GH administrated humanized liver mice. **A** Schematic diagrams illustrating the outline of the study protocol for the 4-day-administration of TO to h-GH-treated humanized liver mice. h-GH administered mice received daily injection of the vehicle or TO from days 10 to 13, and autopsies were performed on day 14. **B** Histological examination using ORO staining (a) and results of quantitative analysis of the ORO-positive area (b). Bar, 100 µm. **C** qRT-PCR results of lipid synthesis-related genes. **D** qRT-PCR results for hGH- and lipid metabolism-related genes. **E** Plasma ALT, AST, and TG levels quantified using the Fuji DryChem System. Each bar represents the mean ± SD. Asterisks above the lines indicate significance among groups. $*P < 0.05$, $**P < 0.01$ (Dunnet test)

and *human fatty acid synthase* (*h-FASN*) was examined by qRT-PCR after 14 days. These results clearly indicate that these lipogenesis-related genes were activated in TO901317-treated animals after 2 weeks of h-GH administration (Fig. 3C). Additionally, the effect of TO901317 treatment on GH- or lipid metabolism-related genes was analyzed. The expression of *h-GHR* partially decreased, but of *h-IGF1* did not change. Although the expression of *h-ABCA1* was increased by the administration of 100 mg/kg TO901317, that of *h-CPT1a* was reduced (Fig. 3D). Moreover, TO901317 treatment increased plasma ALT and AST activities and TG levels (Fig. 3E). These results indicate that h-GH-administered chimeric mice can be utilized as an animal model for DIFLD research for both histological blood biochemical and gene expression analyses.

Discussion

The evaluation system for DIFLD, a specific form of DILI, could help improve the accuracy of predicting human DILI. Although chimeric mice mimic human liver function (Katoh et al. 2005; Kisoh et al. 2021; Okumura et al. 2007), this mouse model spontaneously develops a fatty liver after transplantation because of the incompatibility between m-GH and h-GHR (Tateno et al. 2011). Herein, we propose an optimized dosage of h-GH (0.25 mg/kg) that represents the reduction in lipids and physiological blood h-GH levels to reproduce physiological conditions in the human liver. This novel h-GH dosage will be useful for constructing a highly accurate DIFLD evaluation system.

Additionally, to investigate whether the optimized GH treatment modulated the expression levels of GH-related genes (*h-IGF1*, *h-SOCS2*, and *h-GHR*) in chimeric mice more closely resemble those in humans, the results of qRT-PCR (Fig. 2C) were compared with those of the microarray analysis of the relative expression levels in humans to chimeric mice, which we previously reported (Supplementary Table 1) (Tateno et al. 2011). Consistent with the results of blood h-GH, the expression levels of *h-IGF1* and *h-SOCS2* in chimeric mice without h-GH administration were lower than those in humans in the microarray analysis (Supplementary Table 1) and were found to be up-regulated after h-GH treatment in this study. In particular, the relative

expression levels to chimeric mice without h-GH suggested that 0.25 mg/kg h-GH treatment up-regulated the expression levels of *h-IGF1* (23.5 ± 2.4) (Fig. 2C(a)) and *h-SOCS2* (203.6 ± 66.1) (Fig. 2C(b)) closer to those in human (*h-IGF1*:23.6 or 8.3, *h-SOCS2*:87.0) (Supplementary Table 1). These results suggest that 0.25 mg/kg h-GH treatment modulates GH signaling levels in chimeric mice closer to those in humans.

Although the effectiveness of GH has been demonstrated, the regulatory mechanisms of lipogenesis in humans remain unclear. In this study, we analyzed the expression levels of *h-SCD* (lipogenesis), *h-ABCA1* (lipid efflux), and *h-CPT1a* (lipid oxidation) using qRT-PCR. Our results indicated that h-GH treatment suppressed *h-SCD* mRNA expression (≥ 0.25 mg/kg h-GH) and activated *h-ABCA1* and *h-CPT1* mRNA expressions (0.5 and 1.0 mg/kg h-GH) (Fig. 2C(d–f)). The results of the analysis of our previous microarray data also suggested that h-GH-untreated chimeric mice showed higher *h-SCD* expression and lower *h-ABCA1* expression compared with humans (Supplementary Table 1). These results suggest that h-GH-administered chimeric mice represent the biological dynamics of lipid synthesis and lipid metabolism closer to those in humans, particularly with 0.25 mg/kg GH administration. Consistent with our results, previous studies have shown that liver-specific GHR-knockout mice showed upregulation of lipogenesis-related genes, including *m-Scd* (Cordoba-Chacon et al. 2015). IGF1 treatment of HepG2 cells suppressed cholesterol accumulation through the upregulation of *h-ABCA1* (Fukunaga et al. 2018), and GHD model mice showed downregulation of *m-Cpt1* (Sangiao-Alvarellos et al. 2010). These in vivo and in vitro studies have shown that GH/IGF1 signaling increases the expression of ABCA1 and CPT1a, suggesting that the increase in serum GH and IGF1 levels might upregulate the expression levels of *h-ABCA1* and *h-CPT1a* in chimeric mice with 0.5 or 1 mg/kg h-GH administration, although h-GH-untreated chimeric mice showed lower *h-CPT1a* expression compared with humans (Supplementary Table 1). These findings suggest that chimeric mice can be used as animal models to elucidate the regulatory mechanism of lipid metabolism in the human liver through the h-GH signaling pathway.

In addition to the results of the histological observations and gene expression levels, the novel dose treatment (0.25 mg/kg) was able to maintain blood h-GH levels comparable to those of human adults, suggesting that the h-GH treated chimeric mice would potentially replicate human physiological conditions. However, other factors may also be involved in the spontaneous development of fatty liver in humanized liver chimeric mice. For instance, interleukin-6 (IL-6) regulates lipid metabolism, as shown by the steatosis in the liver of IL6-KO mice (Wallenius et al. 2002). A recent study also showed that the transplantation of m-IL6R–expressing human hepatocytes or h-IL6 supplementation reduced the number of lipid droplets in chimeric mouse livers (Carbonaro et al. 2023). Additionally, JAK/STAT signaling, a major pathway of GH and IL6 signaling, is regulated by multiple factors such as hormones, interferons, interleukins, and colony-stimulating factors (Hu et al. 2021). Taken together, exogenous treatment with these cytokines or growth factors may play an important role in improving the physiological conditions of h-GH-treated chimeric mice.

Based on these aforementioned results, in vivo evaluation of human DIFLD was tested with 0.25 mg/kg h-GH-administered chimeric mice. The present study showed that hepatic lipogenesis was activated by TO901317, suggesting that h-GH-administered chimeric mice could be useful in the evaluation of DIFLD. In previous studies, DIFLD in mice occurred within 4 days of the administration of test substances, including TO901317 (10 mg/kg/day) (Grefhorst et al. 2002). In this study, the administration of TO901317 for 4 days also induced liver steatosis in chimeric mice treated with h-GH (Fig. 3). These results suggest that the evaluation of human DIFLD with h-GH-administered chimeric mice could also be carried out in a timeframe similar to that of a normal mouse model (for example, 4 days). Furthermore, a long-term experiment showed that the administration of TO901317 for 7 days to chimeric mice treated with h-GH for 4 weeks was also able to induce lipid accumulation to the same extent as short-term administration (Supplementary Fig. 1). Long-term h-GH administration to chimeric mice would be useful for the long-term assessment of DIFLD. For instance, it has been shown that 2 weeks administration of ursodeoxycholic acid increases the expression of *SCD* and the amount of TG in the liver in patients with NAFLD, while ursodeoxycholic acid decreases these levels in mice (Mueller et al. 2015). h-GH-administered chimeric mice may contribute to DIFLD evaluation and mechanistic elucidation of drugs that induce human-specific lipid accumulation, such as ursodeoxycholic acid.

Additionally, TO901317 regulates the expression of lipid synthesis-related genes (Chisholm et al. 2003; Grefhorst et al. 2002; Kim et al. 2021), GH (Zadjali et al. 2011), and lipid metabolism (Ide et al. 2003; Tamehiro et al. 2007) related genes. The lipid synthesis-related genes were increased by TO901317 treatment (Fig. 3C). Although TO901317 inhibits GH signaling in rat hepatocytes by activating *SREBP1* and suppressing JAK/STAT signaling (Zadjali et al. 2011), in this study, the expression level of *h-IGF1* did not decrease with TO901317 treatment (Fig. 3D, Supplementary Fig. 1D). In addition, expression levels of *h-ABCA1* (an LXR target gene and lipid metabolism-related transporter) and *h-CPT1a* (a lipid metabolism-related gene) did not increase with 0.25 mg/kg hGH treatment (Fig. 2C(e, f)), suggesting that the effect

of h-GH on the expression of these genes was minor in the GH-treated chimeric mice. However, consistent with previous studies (Ide et al. 2003; Tamehiro et al. 2007), *h-ABCA1* and *h-CPT1a* levels increased and decreased with TO901317 treatment, respectively (Fig. 3D, Supplementary Fig. 1D). The activation of *h-ABCA1* also indicates lipid excretion, in addition to LXR activation, suggesting a dual role for LXR in improving lipid metabolism and inducing fatty liver (Zhang et al. 2012). These results suggest that TO901317 regulates lipid synthesis and metabolism, resulting in the induction of lipid accumulation through LXR activation, without inhibiting GH signaling in chimeric mouse livers, probably due to species differences in hepatocytes. Furthermore, in hGH-treated chimeric mice, TO901317 induced lipid accumulation independent of hGH signaling, suggesting that DIFLD could also be assessed in this model with other agents.

Furthermore, our results regarding the h-GH dose-dependent reduction of lipid droplets suggest a correlation between GH levels and lipid metabolism in humans. Chimeric mice, similar to patients with GHD, lack h-GH, and the number of lipid droplets in the liver was reduced by the 0.25 mg/kg h-GH dosage, which is within the range of somatropin dosage for the patients (approximately 0.021 ~ 0.47 mg/kg/day). In addition, aging is known to induce a reduction in GH levels and fatty liver in humans (Sumida et al. 2015). In this study, the GH dosage of less than 0.25 mg/kg (i.e. 0.1 mg/kg) did not sufficiently reduce lipid droplets in the liver, which is consistent with the finding that SCD gene expression in the liver was significantly reduced by at least 0.25 mg/kg. Collectively, our present study suggests that chimeric mice with 0.25 mg/kg or less h-GH treatment would represent the mechanism of lipid metabolism by h-GH treatment in patients with GHD and the physiological conditions associated with aging in humans.

In summary, we have developed a new study model using h-GH-administered chimeric mice. Under optimized activation of h-GH signals, this model appears to be a more reliable in vivo platform for studying the regulatory mechanisms of lipogenesis and evaluating DIFLD in humans. Additionally, in an h-GH dose-dependent manner, chimeric mice showed a wide range of h-GH levels, including those in humans, which would be a suitable model for the functional analysis of h-GH in humans with GHD or aging.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00204-025-03986-5.

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Data availability Supplementary data are available online.

Declarations

Conflict of interest Sho Morioka, Yuji Ishida, Suzue Furukawa, Yuko Ogawa, Chise Tateno are employees of PhoenixBio Co., Ltd. Seigo Sanoh received financial support from PhoenixBio Co. Ltd. for the collaborative study.

Ethical approval All procedures involving human hepatocytes were approved by the Utilization of Human Tissue Ethical Committee of Phoenix Bio Co., Ltd. All experimental procedures involving animals were conducted in accordance with the guidelines provided by the Proper Conduct of Animal Experiments (June 1, 2006; Science Council of Japan). All procedures were approved by the Laboratory Animal Ethics Committee of Phoenix Bio Co., Ltd.

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