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Treatment with a farnesyltransferase inhibitor improves survival in mice with a Hutchinson–Gilford progeria syndrome mutation

Shao H. Yang, Xin Qiao, Loren G. Fong, Stephen G. Young *

Department of Medicine/Division of Cardiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

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Abstract

Hutchinson–Gilford progeria syndrome (HGPS) is a progeroid syndrome characterized by multiple aging-like disease phenotypes. We recently reported that a protein farnesyltransferase inhibitor (FTI) improved several disease phenotypes in mice with a HGPS mutation ($Lmna^{HG/+}$). Here, we investigated the impact of an FTI on the survival of $Lmna^{HG/+}$ mice. The FTI significantly improved the survival of both male and female $Lmna^{HG/+}$ mice. Treatment with the FTI also improved body weight curves and reduced the number of spontaneous rib fractures. This study provides further evidence for a beneficial effect of an FTI in HGPS.

Keywords: Progeria; Aging; Protein farnesyltransferase inhibitor; Knock-in mice

1. Introduction

Children with Hutchinson–Gilford progeria syndrome (HGPS) appear normal at birth but then exhibit retarded growth, osteolytic lesions in bones, osteoporosis, alopecia, loss of subcutaneous fat, and ultimately occlusive vascular disease and premature death [1]. HGPS is caused by a *LMNA* point mutation that alters the splicing of the prelamin A pre-mRNA, leading to a 50-amino acid internal deletion within prelamin A [2,3]. The mutant prelamin A protein in HGPS, often called progerin, is farnesylated and methylated at a carboxyl-terminal *CaaX* motif [4,5]. Progerin is targeted to the nuclear rim, where it interferes with the integrity of the nuclear lamina and leads to misshapen nuclei [2]. Several studies have shown that the frequency of misshapen nuclei in HGPS cells is reduced by treating cells with a protein farnesyltransferase inhibitor (FTI) [4,6–11]. Interestingly, the FTIs do not appear to have significant adverse effects on the growth of wild-type cells [11].

The FTI studies in cultured cells prompted interest in testing FTIs in mouse models of HGPS. Yang *et al.* [12] created a gene-targeted HGPS allele ($Lmna^{HG}$) and showed that heterozygous mice ($Lmna^{HG/+}$) develop a host of progeria-like disease phenotypes (*e.g.*, normality at birth followed by slow growth, osteolytic lesions, spontaneous rib fractures, a reduction in subcutaneous fat, and premature death). Treatment of $Lmna^{HG/+}$ mice with an FTI (ABT-100) improved a number of bone phenotypes, including the number of spontaneous rib fractures [12].

The results of the initial FTI study in $Lmna^{HG/+}$ mice were encouraging, but we firmly believed that additional studies in mice were essential before contemplating human clinical trials, in part because the initial FTI study did not assess the impact of the drug on the survival of $Lmna^{HG/+}$ mice [12]. Also, the initial FTI study involved relatively small numbers of mice [12]. For these reasons, we set up an independent trial of FTI treatment in $Lmna^{HG/+}$ mice, focusing on whether the drug would improve survival and other hallmark phenotypes of progeria.

2. Materials and methods

Male and female $Lmna^{HG/+}$ and $Lmna^{+/+}$ mice were bred by mating female C57BL/6 mice with male chimeric mice (generated with $Lmna^{HG/+}$ mouse embryonic stem cells). Because all of the mice in this study were bred from chimeras, the $Lmna^{HG/+}$ and $Lmna^{+/+}$ mice were genetically identical (except for the targeted mutation). Genotyping was performed by PCR with genomic DNA prepared from biopsies of the tail [6]. The mice were fed a chow diet and housed

* Corresponding author. 695 Charles E. Young Dr. South, Los Angeles, CA 90095, USA. Tel.: +1 310 825 4422; fax: +1 310 206 0865.
E-mail addresses: lfong@mednet.ucla.edu (L.G. Fong), sgyoung@mednet.ucla.edu (S.G. Young).

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in a virus-free barrier facility with a 12-h light–dark cycle. UCLA's Animal Research Committee approved all procedures.

We administered an FTI, ABT-100 [13], to groups of 8–13 male and female *Lmna*$^{HG/+}$ mice and 7–16 male and female *Lmna*$^{+/+}$ mice. ABT-100 was mixed in drinking water containing 0.4% hydroxy methyl propyl cellulose and 1.0% ethanol at a concentration of 0.3 mg/ml, so as to deliver an approximate dose of 39 mg/kg/day. The vehicle consisted of drinking water with 0.4% hydroxy methyl propyl cellulose and 1.0% ethanol. The FTI was initiated at 4 weeks of age and was continued for up to 45 weeks of age (when the last of the *Lmna*$^{HG/+}$ mice died). Previous studies have revealed that this dosage of ABT-100 (~39 mg/kg/day) yields mean plasma ABT-100 concentrations of approximately 0.7 μg/ml [12,14]. This FTI dosage did not elicit liver pathology, as judged by routine histological studies. In one series of experiments, we administered a higher concentration of ABT-100 to *Lmna*$^{+/+}$ (n=14) and *Lmna*$^{HG/+}$ mice (n=10), so as to deliver 117 mg/kg/d.

The accumulation of prelamin A in liver was assessed by western blots. Liver samples were collected and frozen in liquid nitrogen, and urea-soluble extracts were prepared and analyzed by SDS-polyacrylamide gel electrophoresis and western blotting. The antibody dilutions were 1:6000 for a rabbit anti-prelamin A antiserum [6,7], 1:400 for a goat anti-lamin A/C antibody (Santa Cruz Biotechnology), 1:500 for a mouse anti-HDJ-2 antibody (NeoMarkers), 1:1000 for a goat anti-actin IgG (Santa Cruz Biotechnology), 1:6000 for anti-goat IgG-HRP antibody (Santa Cruz Biotechnology), 1:6000 for anti-rabbit IgG-HRP antibody (Santa Cruz Biotechnology), and 1:6000 for HRP-labeled anti-mouse IgG (Amersham Biosciences). Antibody binding was detected with the ECL Plus chemiluminescence system (Amersham) and exposure to X-ray film.

Body weights of *Lmna*$^{+/+}$ and *Lmna*$^{HG/+}$ mice were assessed weekly. Body weight curves were compared with repeated-measures ANOVA and the log rank test. The number of surviving male and female mice was recorded weekly and expressed as a percentage of the total number of mice. The significance of differences was determined by the Kaplan–Meier method.

At the time of death, the number of spontaneous rib fractures in *Lmna*$^{HG/+}$ mice was documented. After opening the thoracic cavity and removing the heart and lungs, the interior of the thorax was photographed with a digital camera, and rib fractures were counted. The number of rib fractures in FTI- and vehicle-treated *Lmna*$^{HG/+}$ mice was compared with a two-tailed Student's t test.

3. Results

We administered an FTI, ABT-100 (39 mg/kg/day) [13], to groups of 8–13 male and female *Lmna*$^{HG/+}$ mice and 7–16 male and female *Lmna*$^{+/+}$ mice. As expected, the FTI treatment interfered with the biogenesis of mature lamin A and resulted in an increased amount of nonfarnesylated prelamin A, as judged by western blots on liver extracts from FTI-treated mice (Fig. 1). The FTI treatment also blocked the farnesylation of HDJ-2, resulting in the appearance of nonfarnesylated HDJ-2 (Fig. 1).

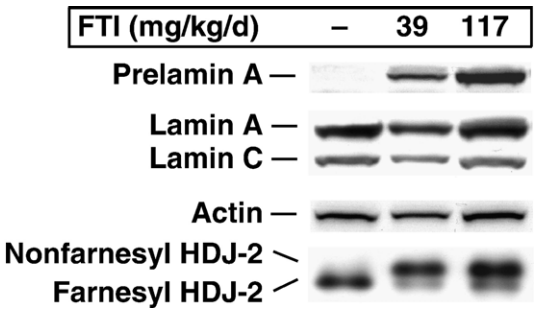

Fig. 1. Western blot analysis of liver extracts. FTI treatment of *Lmna*$^{+/+}$ mice leads to the appearance of nonfarnesylated HDJ-2 (with an antibody against HDJ-2) in liver extracts. FTI treatment also leads to the appearance of nonfarnesylated prelamin A (as judged by a western blot with a prelamin A-specific antibody). However, the amount of prelamin A accumulation was small, as judged by a western blot with an antibody against lamin A/C (prelamin A is a faint shadow above a large lamin A band). The FTI did not reduce the levels of mature lamin A.

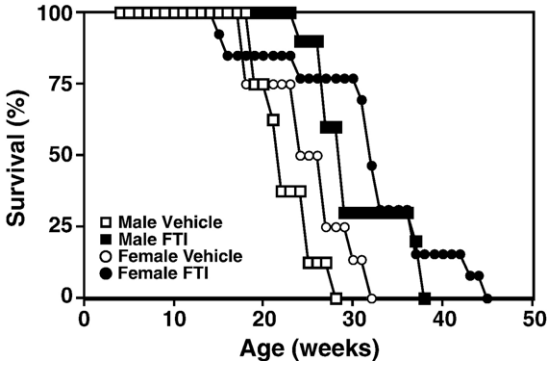

Fig. 2. Kaplan–Meier survival plots for *Lmna*$^{HG/+}$ mice treated with an FTI or vehicle alone. Male *Lmna*$^{HG/+}$ mice on vehicle (n=8); male *Lmna*$^{HG/+}$ mice on FTI (n=10); female *Lmna*$^{HG/+}$ mice on vehicle (n=8); female *Lmna*$^{HG/+}$ mice on FTI (n=13).

Kaplan–Meier survival curves revealed that FTI treatment significantly improved survival in both male (P=0.05) and female *Lmna*$^{HG/+}$ mice (P=0.0027) (Fig. 2), extending the median survival by 6–8 weeks.

FTI treatment improved body weight curves in male (P<0.0001) and female *Lmna*$^{HG/+}$ mice (P<0.0001) (Fig. 3A and B). The FTI treatment was well tolerated in both *Lmna*$^{+/+}$ and *Lmna*$^{HG/+}$ mice. In previous studies [12,14], we found a small but significant reduction in body weight in the *Lmna*$^{+/+}$ mice treated with an FTI. We observed a slight trend towards reduced weight gain in FTI-treated male and female *Lmna*$^{+/+}$ mice in this study (Fig. 3A and B), but these differences did not achieve statistical significance.

The FTI treatment significantly reduced the number of spontaneous rib fractures in *Lmna*$^{HG/+}$ mice (P<0.0001) (Fig. 3C). This was the case in both male and female *Lmna*$^{HG/+}$ mice (P<0.0001 in both groups).

We attempted to deliver higher doses of the FTI to both *Lmna*$^{+/+}$ (n=14) and *Lmna*$^{HG/+}$ mice (n=10), with the hope of achieving greater improvements in disease phenotypes in *Lmna*$^{HG/+}$ mice. However, increasing the concentration of ABT-100 in the drinking water (so as to deliver 117 mg/kg/d) was toxic, even in wild-type mice. During the first week on the higher FTI dosage, 58% of the FTI-treated mice lost >10% of their body weight; by the end of the second week, 74% of the mice had lost >10% of their body weight. During the first 2 weeks of the higher dose of FTI, five mice died (three *Lmna*$^{+/+}$ mice and two *Lmna*$^{HG/+}$ mice). The mice that died had necrosis of the liver. [Finding liver toxicity with the higher dose of ABT-100 was not surprising; liver toxicity with high doses of the drug had been reported by the manufacturer in the MSDS (Material Safety Data Sheet).]

4. Discussion

This study provides additional support for a favorable effect of FTI therapy in *Lmna*$^{HG/+}$ mice. Importantly, we found that