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Poster for you

Pegylated interferon-alpha treatment potently reduces all HDV markers in primary human hepatocytes undergoing cell division *in vivo*

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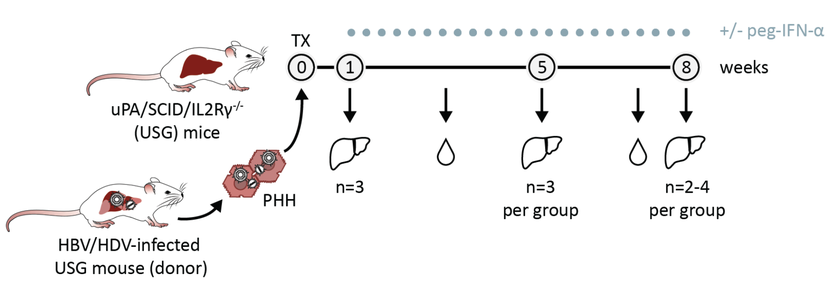
Introduction/Summary

- The endogenous interferon (IFN) response is not sufficient to abrogate hepatitis D virus (HDV) replication nor to inhibit cell division-mediated spread of HDV *in vivo* (*Giersch, Gut 2019*).
- Treatment with pegylated interferon-alpha (pegIFNα) significantly reduced both HDV viremia and intrahepatic HDV markers in humanized mice stably infected with both HDV and hepatitis B virus (HBV) (*Giersch, JHEPRep 2023*).
- *In vitro,* IFNα primarily exhibits HDV antiviral activity in hepatoma cells undergoing cell division (*Zhang, J.Hepatol 2022*).

Aim

Investigate the impact of cell division and pegIFNα treatment on HDV replication in the human liver chimeric mouse model using an experimental setting enabling the proliferation of HBV/HDV infected primary human hepatocytes (PHH).

Methods



- uPA/SCID/IL2Rγ$^{-/-}$ (USG) mice (n=16) were transplanted (TX) with PHHs isolated from a mouse previously reconstituted with PHH and stably infected with HBV and HDV (HBV viremia 1.7×10^9 copies/mL; HDV 5.3×10^8 copies/mL).
- Virological markers, cell proliferation, and IFN responses were analyzed by qPCR, ELISA, and immunofluorescence.
- PegIFNα treatment (25 ng/g biweekly s.c.) was administered from week 1 to 8 to 6/13 mice to assess its impact on HDV, HBV, and cell proliferation.

Conclusion

In line with previous *in vitro* and *in vivo* studies, HDV persists during proliferation and spreads among human hepatocytes undergoing cell division. This study shows that pegIFNα does not hinder PHH proliferation *in vivo*. However, treatment with pegIFNα in an environment supporting cell proliferation potently suppressed HDV.

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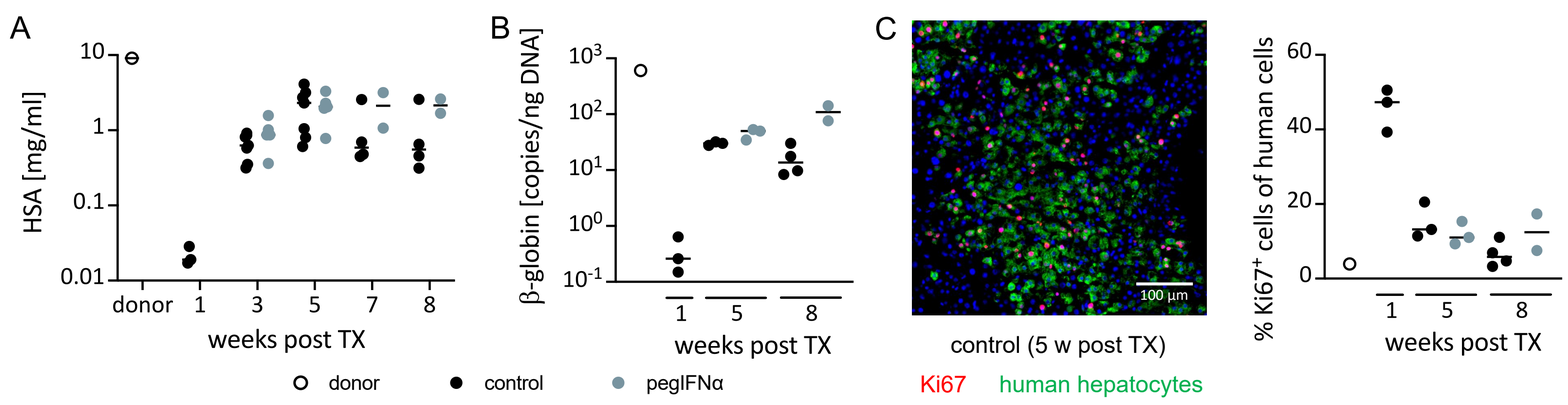
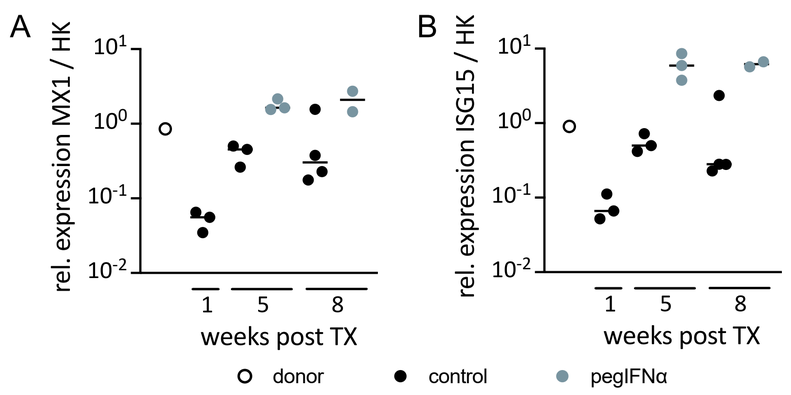
Results

I. STRONG PROLIFERATION OF HBV/HDV-INFECTED PHH FOLLOWING TX



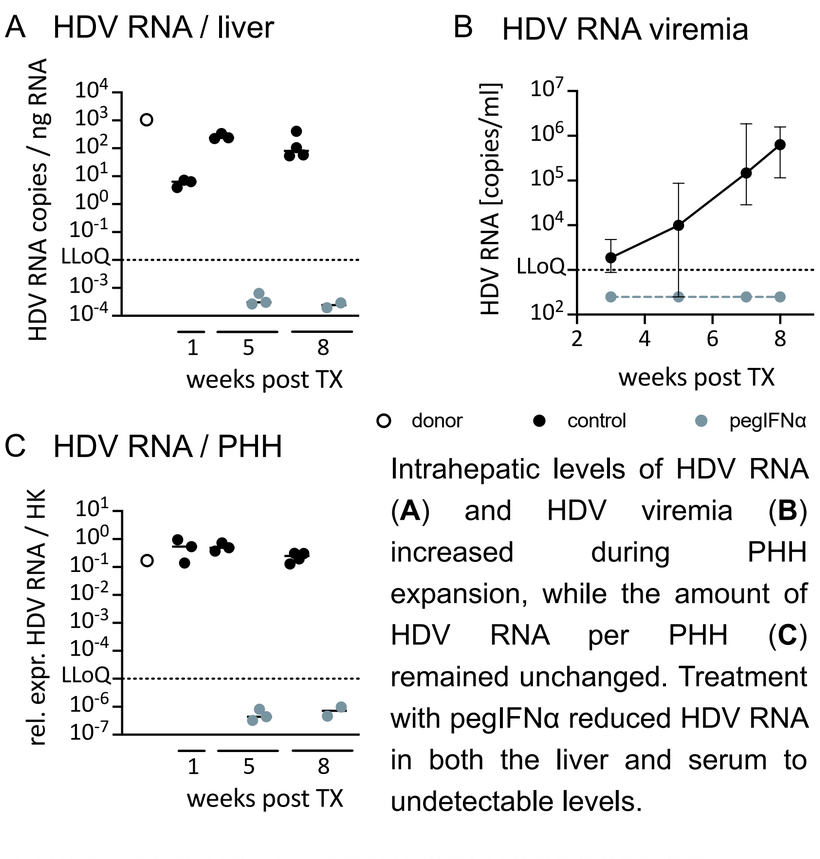
Following serial TX of HBV/HDV infected PHH, strong cellular proliferation was observed, as demonstrated by the increase of human serum albumin (2Log_{10} HSA increase) (**A**), human genome equivalents (β-globin) (**B**), and the cellular proliferation marker (Ki67) (**C**). Of note, PHH expansion was not hindered by pegIFNα treatment.

II. ISG EXPRESSION WAS INDUCED FOLLOWING TX



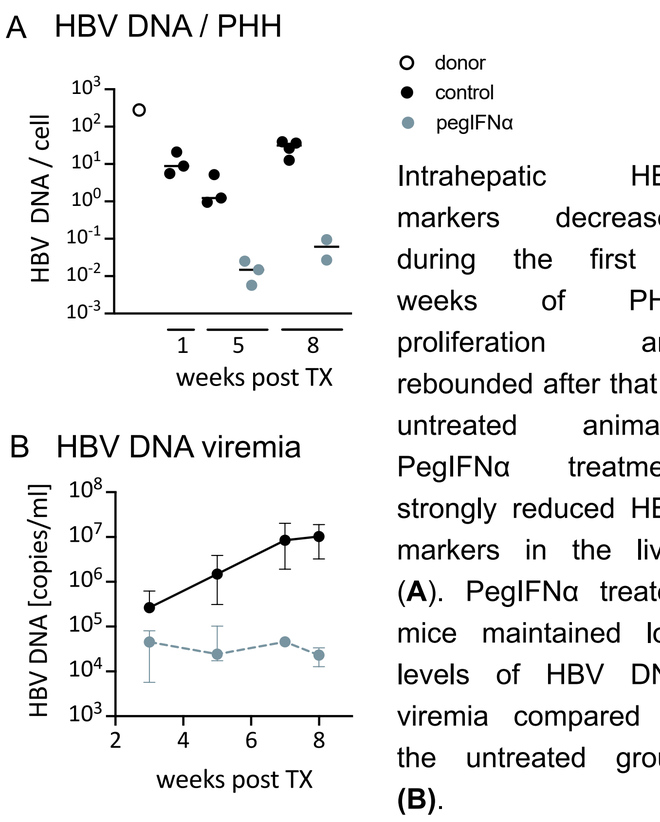
Expression of human interferon-stimulated genes (ISGs) MX1 (**A**) and ISG15 (**B**) was enhanced in mice chronically infected with HBV/HDV, such as in the donor mouse (*Giersch, J.Hepatol 2015*). Expression levels decreased transiently in the first week post PHH transplantation but were again enhanced in the following weeks. ISG expression was further increased in mice treated with pegIFNα.

III. HDV RNA CHANGES IN LIVER AND BLOOD



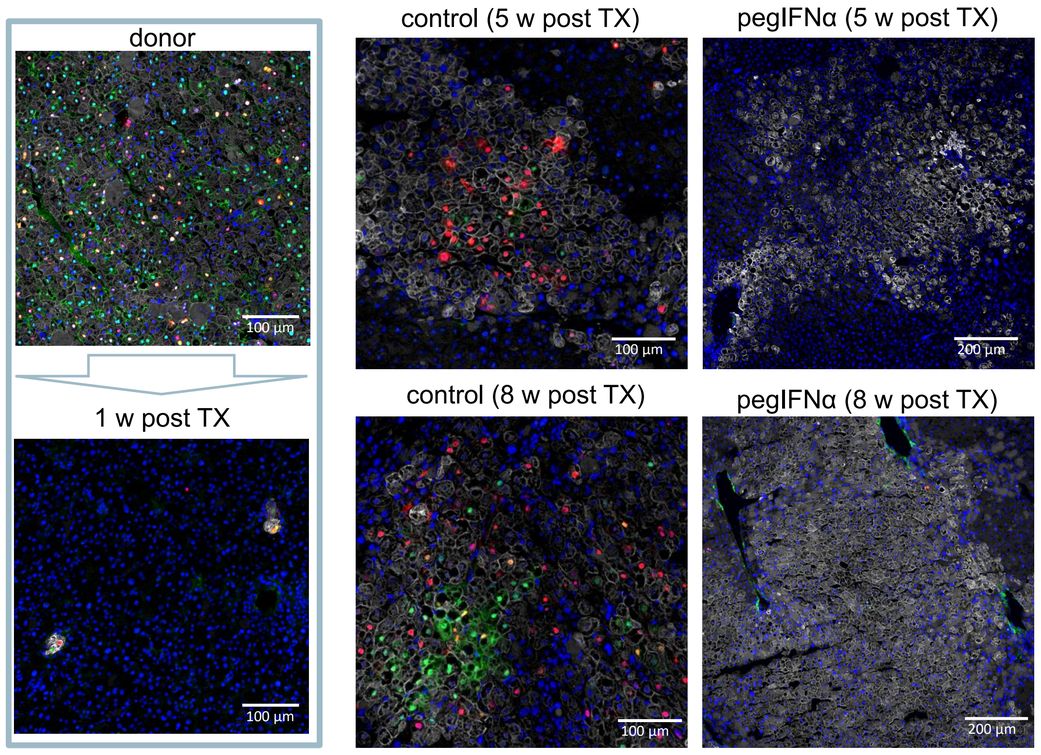
Intrahepatic levels of HDV RNA (**A**) and HDV viremia (**B**) increased during PHH expansion, while the amount of HDV RNA per PHH (**C**) remained unchanged. Treatment with pegIFNα reduced HDV RNA in both the liver and serum to undetectable levels.

IV. HBV CHANGES IN LIVER AND BLOOD



Intrahepatic HBV markers decreased during the first 5 weeks of PHH proliferation and rebounded after that in untreated animals. PegIFNα treatment strongly reduced HBV markers in the liver (**A**). PegIFNα treated mice maintained low levels of HBV DNA viremia compared to the untreated group **(B)**.

V. HDAg AND HBcAg IMMUNOFLUORESCENCE



Consistent with previous studies (*Giersch, Gut 2019*), HDV spread through cell division, leading to higher amounts of Hepatitis Delta Antigen (HDAg)-positive PHH, while intrahepatic HBV markers decreased in the first 5 weeks, resulting in an increased proportion of Hepatitis B core Antigen (HBcAg)-negative/HDAg-positive PHH. PegIFNα treatment resulted in a strong reduction of HBcAg-positive and undetectable levels of HDAg-positive PHH in the liver.

HDAg HBcAg
human hepatocytes (white)