Repopulation of the immunosuppressed retrorsine-treated infant rat liver with human hepatocytes
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**Background:** We succeeded in developing a human (h-) hepatocyte chimeric mouse model by transplanting h-hepatocytes into the livers of albumin enhancer/promoter-driven urokinase-type plasminogen activator-transgenic severe combined immunodeficiency (SCID) mice. This model mimicked the functions of h-hepatocytes quite well, making it useful for testing drug metabolism and toxicity, and as an animal model susceptible to hepatitis B and C virus infection. However, the small body size and total blood volume of the animals restricted the sampling of blood and bile for biochemical analyses. Difficulties in surgical manipulation resulting from the small body size led us to develop a method of creating chimeric rats with livers repopulated by h-hepatocytes for the purpose of studying h-hepatocytes in vivo. Previously, a retrorsine-treated 2/3 partial hepatectomy rat model was used for synergetic hepatocyte transplantation studies. In this study, we used retrorsine-treated infant rats without performing a partial hepatectomy, because infant hepatocytes have the ability to proliferate actively at levels similar to regenerating hepatocytes after a partial hepatectomy. Because SCID rats do not exist, immunosuppressive drugs were used for xenogeneic hepatocyte transplantation.

**Methods:** Two-week-old rats were treated with retrorsine (10 mg/kg b.w.) 3 days before transplantation, followed by liposome clodronate (10 ml/kg b.w.) and 3.2.3 antibodies (30 µl/rat) for depleting macrophages and NK cells 2 days before transplantation. Rat (r-), h-, or mouse (m-) hepatocytes (5 × 10⁵) were transplanted into the rats via the portal vein. For h- and m-hepatocyte transplantation, the rats were injected with FK506 (1 mg/kg b.w.) daily. Three weeks post-transplantation, the livers were harvested and the repopulation ratios were compared among the r-, h-, and m-hepatocyte-transplanted rats. RT-PCR and immunostaining was used to determine the expression of liver-specific mRNA and proteins in the h-hepatocyte-repopulated livers.

**Results:** Retrorsine treatment inhibited hepatocyte proliferation, resulting in a decrease in liver weight and the induction of megalocytic changes in hepatocytes. Three weeks post-transplantation, the r-hepatocytes were engrafted into the retrorsine-treated rat liver and repopulated it at a ratio of 16.4 ± 6.7%. The h-and m-hepatocytes were also engrafted into the rat liver and the repopulation ratios were 2.5 ± 1.5% and 0.9 ± 0.5%, respectively, 3 weeks post-transplantation. The propagated h-hepatocytes in the rat liver expressed albumin, α1-antitrypsin, HNF4, CYP1A2, 2C9, 2D6, 2E1, and 3A4 mRNA and protein. However, 4 weeks post-transplantation, the rats started to develop ascites and the mortality increased.

**Conclusion:** We established a novel rat model for liver repopulation using retrorsine-treated infant rats without partial hepatectomy. The advantages of this method are that (1) fewer cells are required for transplantation, (2) the preparation time before transplantation is shorter, and (3) a partial hepatectomy is not needed. We concluded that h-hepatocytes were able to engraft and underwent appreciable replication in the rat liver for a minimum of 3 weeks post-transplantation while the rats were treated with an immunosuppressive agent. The development of SCID rats or different immunosuppressive drugs is needed to further improve this method.