

Published in final edited form as:

Xenotransplantation. 1997 November 1; 4(4): 262–266. doi:10.1111/j.1399-3089.1997.tb00191.x.

Correction of congenital hyperbilirubinemia in homozygous Gunn rats by xenotransplantation of hamster livers

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Abstract

The homozygous Gunn_{j/j} rat is an animal model for Crigler-Najjar syndrome in which the lack of the enzyme uridine diphosphoglucuronate-glucuronosyltransferase (UDP-GT) results in congenital unconjugated nonhemolytic hyperbilirubinemia. Because the binding of bilirubin to albumin in plasma varies from species to species, xenotransplantation (XTx) of liver afforded in this model the opportunity to study the interactions between xenoproteins of the donor and bilirubin of the recipient. For this purpose, orthotopic liver transplantation (OLTx) was performed from hamster to adult Gunn_{j/j} rats. No immunosuppression (IS) was given to controls. (Group I, n=5) and to OLTx recipients of syngeneic (Gunn_{j/j} rat) grafts (Group II, n=5), whereas tacrolimus (1 mg/kg/day × 15 days, IM) and cyclophosphamide (8 mg/kg/day × 7 days, IP) were administered to animals receiving hamster xenografts (Group III, n=11). While untreated animals (Group I) died within 7 days (6.8±0.2 days) post-transplantation (Tx), the use however of IS resulted in prolonged (30.2±6.8 days) survival of xenogeneic recipients (Group III) who eventually succumbed to rejection. A precipitous decline in total serum bilirubin (TBili) from pre-operative levels of 5.3±1.0 mg/dL to 0.5±0.2 mg/dL was noted in both Group I and III animals, an observation that sustained itself only in the latter group during the course of their follow-up. The decrease in TBili was also associated with a contemporaneous increase in biliary concentration of conjugated bilirubin. No noticeable reversal of hyperbilirubinemia was however observed in OLTx recipients of syngeneic grafts (Group II). Taken together, these data suggest that hamster albumin and hepatocyte-associated xenoproteins and enzymes involved in the process of membrane transport and glucuronidation of bilirubin, functioned efficaciously after OLTx in Gunn_{j/j} rats, resulting in the reversal of the inborn error of metabolism for the duration of follow-up.

Keywords

Crigler-Najjar syndrome; hyperbilirubinemia; xenotransplantation

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This work was presented at the 14th Annual Meeting of the American Society of Transplant Physicians, May 14–17, 1995, Chicago, Illinois.

Introduction

The generic question of metabolic incongruity associated with Tx of xenogeneic organs has not yet been resolved [1]. We have documented previously that livers transplanted orthotopically across xenogeneic barriers continue to produce proteins of donor-phenotype [2–4]. However, it is not clear if these proteins would interact adequately with proteins or other molecules of the recipient thus mitigating interspecies incompatibilities. In an attempt to address this issue pragmatically, we resorted to transplanting hamster livers into adult Gunn^{jj} rats; an animal model of Crigler-Najjar syndrome, in which due to the absence of enzyme UDP-GT, a rare form of autosomal recessive congenital unconjugated hyperbilirubinemia, is observed [5–7].

Bilirubin-IX α , derived from heme catabolism, is a tetrapyrrole pigment that has no variability among different species [8]. Accordingly, the uptake, conjugation and secretion of rat bilirubin by a transplanted hamster liver would be expected to continue without problems after transplantation. Indeed, Moscioni et al. [9] have demonstrated that human hepatocytes transplanted into athymic homozygous Gunn rat recipients resulted in demonstrable increases in biliary concentration of conjugated bilirubin and a relatively small contemporaneous decrease of serum Tbili. However, bilirubin is mainly bound to albumin in the plasma and this binding varies considerably from species to species [10,11]. Thus, we argued that in an event of optimal interaction between xenogeneic (hamster) albumin with rat bilirubin, livers transplanted from normal hamster should be able to reverse the inborn error of bilirubin metabolism in Gunn^{jj} rats.

Materials and methods

Animals

Male LVG (Golden Syrian) hamsters (120–150 g) and inbred female homozygous Gunn^{jj} (Hsdblu) rats (240–280 g) were used as donors and recipients, respectively. Hamsters were purchased from Charles River Laboratories (Wilmington, MA) and Gunn rats from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The animals were maintained in the specific pathogen-free facilities of the University of Pittsburgh Medical Center and provided with Purina rodent chow and tap water ad libitum.

Liver transplantation

OLTx was performed according to the cuff technique [12,13]. Donor cholecystectomy was carried out at the time of liver grafting and the hepatic artery was not anastomosed. All procedures were performed under methoxyflurane (Pitman-Moore, Mundelein, IL) anesthesia. Autopsy specimens from animals who had either died or were sacrificed during the course of their follow-up were fixed in 10% formalin and stained with hematoxylin and eosin for routine histopathological examination.

Immunosuppression (IS) Protocol

OLTx recipients were immunosuppressed with tacrolimus (FK506, Fujisawa USA, Inc., Dearfield, IL) given at a dose of 1 mg/kg/day \times 15 days, intramuscularly (I.M.) and cyclophosphamide (NEOSAR®, Pharmacia, Inc., Columbus, OH) administered at a dose of 8 mg/kg/day \times 7 days, intraperitoneally (I.P.).

Experimental design

Gunn^{jj} rat recipients of OLTx were divided into the following three groups; Group I, received hamster livers without IS ($n = 5$); Group II, received livers from syngeneic Gunn^{jj} rats without IS ($n = 5$); and Group III, received hamster livers with IS ($n = 11$).

Kinetics of bilirubin

From Gunn j/j rat recipients of hamster OLTx (without IS), serum and bile samples were collected every 6 hr for the first 24 hr for determination of the levels of TBili and conjugated bilirubin in the serum and bile, respectively. Furthermore, in animals in Group III, serum TBili levels were determined at day 3 and 6 and every sixth day thereafter until rejection.

Bile collection

For the kinetics study, a PE-50 polyethylene tube (Intramedic, Becton Dickinson, Rutherford, NJ) was inserted into the bile duct of the hamster liver close to its bifurcation at the hepatic hilum. At the end of the transplant procedure, this tube was externalized through the abdominal wall and subcutaneous tissue to the dorsal surface of the rat where it was drained into a 3 cc Vacutainer (Becton Dickinson, Rutherford, NJ) secured to the skin. At prescribed sampling times, the vacutainer was emptied and new bile was allowed to flow for a period of 30 min, at which time 150–200 μ l was collected. Few ($n=5$) Gunn j/j rat recipients of hamster OLTx (with IS) underwent transverse laparotomy at 2 weeks post-Tx followed by insertion into the distal end of the bile duct of a PE-50 polyethylene tube. The bile was then allowed to flow normally for 1 hr with 300–400 μ l being collected from each rat.

Biochemical analysis

Both total and direct (conjugated) bilirubin levels in serum and bile were determined colorimetrically using pre-defined kits (Sigma Chemical Co, St. Louis, MO). Values were determined spectrophotometrically by measuring the absorbance at an optimal density of 600 nm. The levels of indirect (unconjugated) bilirubin were obtained by calculating the difference between total and direct bilirubin.

Results

Animal survival

Untreated Gunn j/j rat recipients of hamster livers died within 7 days post-Tx (Table 1, Group I). Conversely, prolonged (30 ± 6.8 d) survival was observed when hamster \rightarrow Gunn j/j rat recipients of OLTx were treated with a short course of IS (Table 1, Group m). Autopsy specimens of xenografted livers obtained from Group III animals when examined histopathologically, revealed marked mononuclear infiltration with widespread hepatic necrosis. These findings were consistent with the diagnosis of rejection, which apparently developed after the termination of the short course of IS. As expected, all Gunn j/j rat OLTx recipients of syngeneic (Gunn j/j rat) livers survived for the entire duration of their follow-up, being sacrificed at 2 months post-Tx (Table 1, Group II).

Kinetics of bilirubin metabolism

Within 18 hr of OLTx from hamster in untreated (shown) or treated (not shown) homozygous Gunn j/j rats, a precipitous decline in serum TBili from preoperative levels of 5.3 ± 1.0 to 0.5 ± 0.2 mg/dL was observed (Fig. 1). Over the same period of follow-up, a contemporaneous increase in the biliary concentration of conjugated bilirubin was evidenced (Fig. 1), suggesting that the uptake, conjugation, and secretion of rat bilirubin was proceeding normally in the orthotopically transplanted hamster livers, allowing for the reversal of hyperbilirubinemia.

Owing to the onset of rejection, normal levels of serum TBili could not be sustained in animals in Group I, which showed elevated levels of this pigment after the 3rd post-Tx day. However, in animals in Group III in whom rejection had been delayed by a short course of IS, normobilirubinemia was preserved for up to 24 days post-Tx (Fig. 2). Ongoing rejection

coupled with deteriorating liver function resulted in mild elevation of serum TBili by day 30 post-Tx in Group III animals, an observation sustained until the demise of the animal (Fig. 2). Importantly, no mitigation of hyperbilirubinemia was noted in homozygous Gunn^{j/j} rat OLTx recipients of Gunn^{j/j} rat livers (Fig. 2).

While there was a preponderance of unconjugated bilirubin in the serum of homozygous Gunn^{j/j} rats prior to OLTx, this finding was reversed subsequent to liver xenografting, where within 3 days post-Tx, a precipitous drop in unconjugated and a slight increase in conjugated bilirubin was evidenced (Fig. 3).

Discussion

It has been documented previously that after Tx, proteins and enzymes contained within the liver, retain the phenotype of that of the donor [2,3,14,15]. It is therefore imperative that prior to Tx of organs such as livers, which are involved in diverse physiological and biochemical functions, factors that govern the metabolic compatibilities between the proteins of the donor and proteins or other molecules from the recipient be considered. For Tx across allogeneic barriers, this incongruity does not seem to exist since we have shown that liver-associated inborn errors of metabolism can be corrected effectively by orthotopic replacement of diseased organs with those obtained from human cadaveric donors [16]. However, Tx of livers across xenogeneic barriers may raise the possibility of metabolic incompatibilities necessitating their careful delineation. To address this issue, we transplanted hamster livers into homozygous Gunn rats, in whom the absence of the enzyme UDP-GT results in the manifestation of unconjugated nonhemolytic hyperbilirubinemia [5–7]. Since there is no variability of the bilirubin molecule among different species [8], it is expected that its process of membrane transport and glucuronidation would proceed unabatedly after liver xenotransplantation. However, bilirubin reaches the hepatocytes bound to albumin and a broad variability from species to species has been reported in this interaction [10,11]. Thus, with human albumin as the ligand, the biologic half-life of bilirubin averaged 3.7 hr, whereas with rat albumin it was only 1.5 hr [11]. Unfortunately, there are no reports about the binding to hamster albumin. We reasoned that should selective interaction between hamster albumin and rat bilirubin ensue, Tx of normal hamster livers should reverse the congenital error of bilirubin metabolism in homozygous Gunn rats.

In this study, the reversal of hyperbilirubinemia in homozygous Gunn rats by Tx of normal hamster livers provided indirect evidence that rat bilirubin efficiently bound to hamster albumin and that, as expected, the hamster UDP-GT was capable of conjugating rat bilirubin. The contemporaneous but reciprocal increase in the biliary concentration of conjugated bilirubin suggested that in addition to effective uptake and glucuronidation, protein carriers in hamster livers were capable of efficiently secreting metabolic products of xenogeneic (rat) origin.

The kinetics of these observed biochemical alterations were extremely rapid. After hamster OLTx, the ensuing metabolic changes in Gunn rats resulted in normalization of hyperbilirubinemia within 18 hr post-Tx. While this observation was sustained for almost three weeks in Gunn rats who received a short course of IS, no alteration in serum TBili concentration was noted in recipients of syngeneic (Gunn rat) livers. The marked reduction in the serum levels of unconjugated bilirubin in homozygous Gunn rat recipients of hamster liver was accompanied by a simultaneous increase in the biliary concentration of conjugated bilirubin. Since homozygous Gunn rats lack the enzyme UDP-GT in both hepatic and extra-hepatic sites, it is reasonable to assume that metabolic processing of unconjugated rat bilirubin proceeded exclusively in the orthotopically transplanted hamster liver.

Unlike that observed in other in born errors of metabolism [17–20], hepatic cirrhosis is not a histopathological finding in the Crigler-Najjar syndrome. The unaltered liver function in patients suffering from the latter syndrome therefore raises uncertainties concerning the widespread utility of OLTx as a treatment of choice for palliation of this disease. However, the experimental application of alternative strategies such as hepatocyte [21–25] or small bowel [26] Tx have resulted in less than desirable outcomes. Interestingly, even genetically modified homozygous Gunn rat livers that have been transfected with rat UDP-GT cDNA only partially succeed in reversing hyperbilirubinemia [27]. Our finding of long-term mitigation of hyperbilirubinemia in Gunn rats following (hamster) liver Tx underscores the importance of this procedure for the complete reversal of the disease. This observation is further substantiated by that of Asonuma et al. [28], who, while using a heterotopic (auxiliary) partial liver allograft model, demonstrated that as little as 12% of the liver mass was sufficient to dramatically reduce hyperbilirubinemia in homozygous Gunn rats.

In conclusion, this study has demonstrated the reversal of congenital hyperbilirubinemia in Gunn rats after xenotransplantation of hamster livers, indicating a metabolic compatibility in the processing of rat bilirubin by hamster albumin and hepatocyte proteins involved in the metabolism of this molecule.

Acknowledgments

This work was aided by Project Grant No. DK29961 from the National Institutes of Health, Bethesda, MD.

References

1. Starzl TE, Valdivia LA, Murase N, Demetris AJ, Fontes P, Rao AS, Manez R, Marino IR, Todo S, Thomson AW, Fung JJ. The biological basis of and strategies for clinical xenotransplantation. *Immunol Rev* 1994;141:213. [PubMed: 7868154]
2. Valdivia LA, Lewis JH, Celli S, Bontempo FA, Fung JJ, Starzl TE. Hamster coagulation and serum proteins in rat recipients of hamster xenografts. *Transplantation* 1993;56:489. [PubMed: 8356608]
3. Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M, Kusne S, Rudert WA, Trucco M. Baboon to human liver transplantation. *Lancet* 1993;341:65. [PubMed: 8093402]
4. Celli S, Valdivia LA, Kelly RH, Demetris AJ, Fung JJ, Rao AS, Pan F, Tsugita M, Starzl TE. Functional cooperation of xenoproteins after hamster-to-rat liver transplantation: With particular reference to hamster C3 and secretory component for rat IgA. *Xenotransplantation* 1995;2:46.
5. Roy Chowdhury J, Roy Chowdhury N, Gartner U, Wolkoff AW, Arias IM. Bilirubin diglucuronide formation in intact rats and isolated Gunn rat liver. *J Clin Inv* 1982;69:595.
6. Roy Chowdhury J, Roy Chowdhury N. Conjugation and excretion of bilirubin. *Sem Liv Dis* 1983;3:11.
7. Reichen J. Familial unconjugate hyperbilirubinemia syndromes. *Sem Liv Dis* 1983;3:24.
8. Heirwegh, KPM.; Brown, SB. Bilirubin and its conjugates. In: Heirwegh, KPM.; Brown, SB., editors. *Bilirubin: Chemistry*. Boca Raton, FL: CRC Press, Inc; 1982.
9. Moscioni AD, Roy Chowdhury J, Barbour R, Brown LL, Roy Chowdhury N, Competiello LS, Lahiri P, Demetriou AA. Human liver cell transplantation: Prolonged function in athymic-Gunn and athymic-analbuminemic hybrid rats. *Gastroenterol* 1989;96:1546.
10. Ostrow JD, Schmid R. The protein-binding of C¹⁴ - bilirubin in human and murine serum. *J Clin Invest* 1963;42:1286. [PubMed: 14060402]
11. Snyder AL, Schmid R. The conversion of hematin to bile pigment in the rat. *J Lab Clin Med* 1965;65:817. [PubMed: 14281376]
12. Kamada N, Calne RY. A surgical experience with five hundred thirty liver transplants in the rat. *Surgery* 1983;93:64. [PubMed: 6336859]

13. Valdivia LA, Monden M, Gotoh M, Hasuike Y, Kubota N, Ichikawa T, Okamura J, Mori T. Prolonged survival of liver xenografts from hamster to rat by splenectomy and cyclosporine administration. *Transplantation* 1987;44:759. [PubMed: 3321585]
14. Starzl TE, Marchioro TL, Rowlands DT, et al. Immunosuppression after experimental and clinical homotransplantation of the liver. *Ann Surg* 1964;160:411. [PubMed: 14206848]
15. Kashiwagi N, Groth CG, Starzl TE. Changes in serum haptoglobin and group specific component after orthotopic liver homotransplantation in humans. *Proc Soc Exp Biol Med* 1968;128:247. [PubMed: 4172932]
16. Starzl TE, Demetris AJ, Van Thiel DH. Medical progress: Liver transplantation. *N Engl J Med* (Part I) 1989;321:1014.
17. Esquivel CO, Vincent E, Van Thiel D, Gordon R, Marsh W, Makowka L, Koneru B, Iwatsuki S, Madrigal M, Delgado Millan MA, Starzl TE. Orthotopic liver transplantation for alpha-1-antitrypsin deficiency: an experience in 29 children and 10 adults. *Transplant Proc* 1987;19:3798. [PubMed: 3313926]
18. Esquivel CO, Marino IR, Fioravanti V, Van Thiel D. Liver transplantation for metabolic disease of the liver. *Gastroenterol Clin North Am* 1988;17:167. [PubMed: 3292426]
19. Starzl TE, Zitelli BJ, Shaw BW Jr, Iwatsuki S, Gartner JC, Gordon RD, Malatuck JJ, Fox IJ, Urbach AH, Van Thiel DH. Changing concepts: liver replacement for hereditary tyrosinemia and hepatoma. *J Pediatr* 1985;106:604. [PubMed: 2984396]
20. Malatack JJ, Finegold DN, Iwatsuki S, Shaw BW Jr, Gartner JC, Zitelli BJ, Roe T, Starzl TE. Liver transplantation for type I glycogen storage disease. *Lancet* 1983;1:1073. [PubMed: 6133106]
21. Sutherland DER, Matas AJ, Steffes MW, Simmons RL, Najarian JS. Transplantation of liver cells in an animal model of congenital enzyme deficiency disease: the Gunn rat. *Transplant Proc* 1977;9:317. [PubMed: 405773]
22. Groth CG, Arborth B, Bjorken C, Sundberg B, Lundgren G. Correction of hyperbilirubinemia in the glucuronyltransferase-deficient rat by intraportal hepatocyte transplantation. *Transplant Proc* 1977;9:313. [PubMed: 405772]
23. Vroemen JPAM, Blanckaert N, Buurman WA, Heirwegh KPM, Kootstra G. Treatment of enzyme deficiency by hepatocyte transplantation in rats. *J Surg Res* 1985;39:267. [PubMed: 3928971]
24. Cobourn CS, Makowka L, Falk JA, Falk RE. Allogeneic intrasplenic hepatocyte transplantation in the Gunn rat using cyclosporine A immunosuppression. *Transplant Proc* 1987;19:1002. [PubMed: 3274263]
25. Holzman MD, Rozga J, Neuzil DF, Griffin D, Moscioni AD, Demetriou AA. Selective intraportal hepatocyte transplantation in analbuminemic and Gunn rats. *Transplantation* 1993;55:1213. [PubMed: 8516804]
26. Medley MM, Hooker RL, Rabinowitz S, Holton R, Jaffe BM. Correction of congenital indirect hyperbilirubinemia by small intestinal transplantation. *Am J Surg* 1995;169:20. [PubMed: 7817994]
27. Branchereau S, Ferry N, Myara A, Sato H, Kowai O, Trivin F, Houssin D, Danos O, Heard JM. Correction of bilirubin glucuronyl transferase deficiency in the Gunn rat with gene transfer into the liver via retrovirus vectors. *Chirurgie* 1993;119:642. [PubMed: 7729182]
28. Asonuma K, Gilbert JC, Stain JE, Takeda T, Vacanti JP. Quantitation of transplanted hepatic mass necessary to cure the Gunn rat model of hyperbilirubinemia. *J Ped Surg* 1992;27:298.

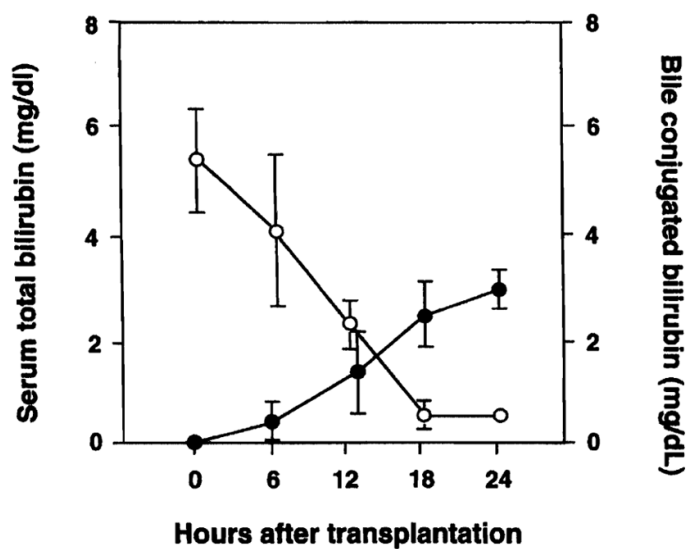


Fig. 1. Changes in the levels of serum TBili (○) and biliary conjugated bilirubin concentration (●) in homozygous Gunn^{j/j} rats following orthotopic hamster liver transplantation ($n=5$). Samples were collected every 6 hr for the first 24 hr post-Tx from non-immunosuppressed recipients.

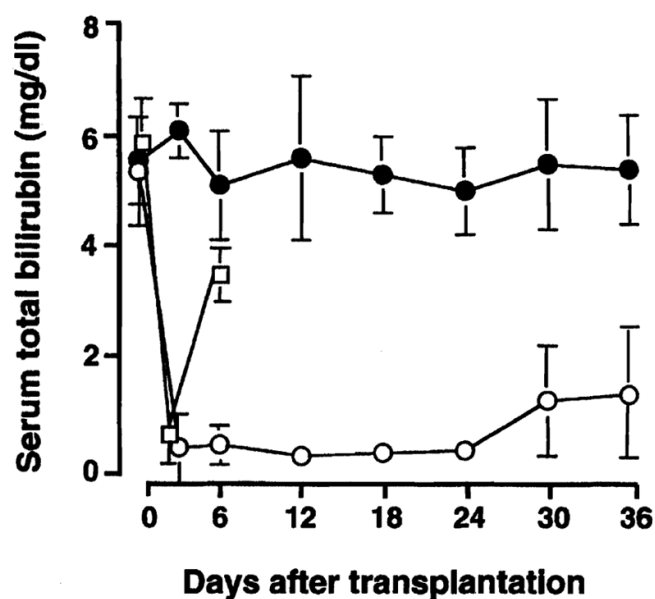


Fig. 2.

Serum TBili levels determined for up to 36 days post-Tx in homozygous Gunn^{j/j} rat OLTx recipients. Group I, hamster→Gunn^{j/j} rat without IS (□); Group II, Gunn^{j/j} rat→Gunn^{j/j} rat without IS (●); Group III, hamster →Gunn^{j/j}rat with IS (○). The details of IS are provided in Materials and Methods.

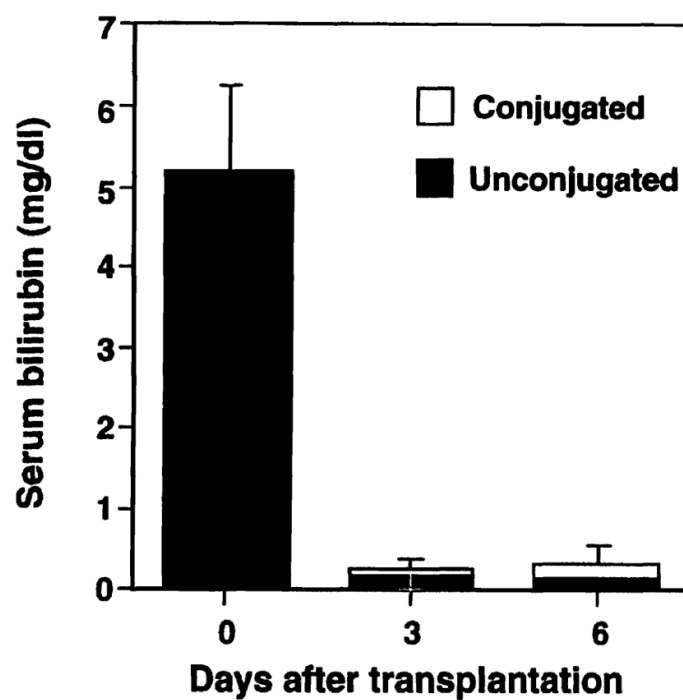


Fig. 3. Changes in serum conjugated (□) and unconjugated (■) bilirubin levels at 2 weeks after hamster liver XTx in homozygous Gunn^{j/j} rats who were treated additionally with a short course of IS.

Table 1

Survival of homozygous Gunn(*j/j*) rat y recipients of hamster liver xenografts following a short course of immunosuppression with tacrolimus and cyclophosphamide

Group	Donor→Recipient	Treatment	Xenograft survival days	Mean ± SD
I	Hamster→Gunn rat _{j/j}	None	6,7,7,7,7	6.8 ± 0.2
II	Gunn rat _{j/j} →Gunn rat _{j/j}	None	> 50 × 5	>50
III	Hamster→Gunn rat _{j/j}	Tacrolimus ^a + Cyclophosphamide ^b	21,22,23,27,30,31,31,31,35,39,42	30.2 ± 6.75

^aTacrolimus was given in a dose of 1 mg/kg/day, I.M., for 14 days.

^bCyclophosphamide was given in a dose of 8 mg/kg/day, i.p. for 7 days.